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(21) International Application Number: PCT/EP90/02154 (22) International Filing Date: 12 December 1990 (12.12.90) (30) Priority data: 89403493.3 14 December 1989 (14.12.89) EP (34) Countries for which the regional or international application was filed: AT et al. (71) Applicant (for all designated States except US): HENNING BERLIN GMBH CHEMIE- UND PHARMAWERK [DE/DE]; Komturst. 58-62, D-1000 Berlin 42 (DE). (72) Inventors; and (75) Inventors/Applicants (for US only) : PARMENTIER, Marc [BE/BE]; Avenue Minerve 21 Bte 81, B-1190 Brussels (BE). LIBERT, Frederic [BE/BE]; Brusselbaan 115, B-1600 St-Pieters-Leeuw (BE). DUMONT, Jacques [BE/BE]; Chemin du Chêne-au-Renard 32, B-1328 Ohain (BE). VASSART, Gilbert [BE/BE]; Avenue Lambeau 113, B-1200 Brussels (BE).	(74) Agent: ANDRAE, Steffen; Patentanwälte Andrae, Flach, Haug, Kneissl, Balanstr. 55, D-8000 München 90 (DE). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK, DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published <i>Without international search report and to be republished upon receipt of that report.</i>																																																																																											
(54) Title: POLYPEPTIDES HAVING THYROTROPIN-RECEPTOR ACTIVITY, NUCLEIC ACID SEQUENCES CODING FOR SUCH RECEPTORS AND POLYPEPTIDES, AND APPLICATIONS OF THESE POLYPEPTIDES (57) Abstract The invention concerns a polypeptide possessing thyrotropin receptor activity, characterised in that it comprises the amino acid sequence (shown in Fig. 11), or a fragment thereof, or an amino acid sequence derived from this sequence by substitution or deletion of any of the amino acid residues (indicated in Fig. 11), or by insertion of additional amino acid residues. <div style="text-align: center;"> Comparison of human and dog TSH receptor sequences </div> <table border="0" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 15%;"></td> <td style="width: 10%; text-align: center;">-20</td> <td style="width: 10%; text-align: center;">1</td> <td style="width: 10%; text-align: center;">20</td> <td style="width: 10%; text-align: center;">40</td> </tr> <tr> <td>Human TSHR</td> <td>MRPADLLQLVLLDLPRDLGGMGCSPPCEHQEEDFRVTKDQIRPSLPSTDTLALI</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Dog TSHR</td> <td>PP H A A S K P D H T F</td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td style="text-align: center;">60</td> <td style="text-align: center;">80</td> <td style="text-align: center;">100</td> </tr> <tr> <td>Human TSHR</td> <td>ETHLRTIPSHAFSNLPSNLSRIYVSIDLTLQQLSHSPYHLSKVTHIEIRNTRNLTYYIDPD</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Dog TSHR</td> <td>Q K R L A R M S S</td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td style="text-align: center;">120</td> <td style="text-align: center;">140</td> <td style="text-align: center;">160</td> </tr> <tr> <td>Human TSHR</td> <td>ALKELPLLKFLGIPNTGLKHPDLTKVYSTDIFFILEITDNPYMTSIPVNAFQGLCNETL</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Dog TSHR</td> <td>GV V V A A</td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td style="text-align: center;">180</td> <td style="text-align: center;">200</td> <td style="text-align: center;">220</td> </tr> <tr> <td>Human TSHR</td> <td>TLKLYNNGFTSVQGYAFHGTLDVAVYLNKNKYLTVIDKDAFGGVYSGPSLLDVSQTSVTA</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Dog TSHR</td> <td>I H SA T Y</td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td style="text-align: center;">240</td> <td style="text-align: center;">260</td> <td style="text-align: center;">280</td> </tr> <tr> <td>Human TSHR</td> <td>LPSKGLKELKELIARNTNTLKLPLSLSLFLHLTRADLSYPHCCAFKNQKKIRGILESIM</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Dog TSHR</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td style="text-align: center;">300</td> <td style="text-align: center;">320</td> <td style="text-align: center;">340</td> </tr> <tr> <td>Human TSHR</td> <td>CHESHSQSLRQRKSVNALNSPLHQEYEEENLGDSTVGYREKSKFQDTHNNAHYTVFFEEQE</td> <td></td> <td></td> <td></td> </tr> <tr> <td>Dog TSHR</td> <td>IR T G PD Y HA DN Q DS S</td> <td></td> <td></td> <td></td> </tr> </table>				-20	1	20	40	Human TSHR	MRPADLLQLVLLDLPRDLGGMGCSPPCEHQEEDFRVTKDQIRPSLPSTDTLALI				Dog TSHR	PP H A A S K P D H T F						60	80	100	Human TSHR	ETHLRTIPSHAFSNLPSNLSRIYVSIDLTLQQLSHSPYHLSKVTHIEIRNTRNLTYYIDPD				Dog TSHR	Q K R L A R M S S						120	140	160	Human TSHR	ALKELPLLKFLGIPNTGLKHPDLTKVYSTDIFFILEITDNPYMTSIPVNAFQGLCNETL				Dog TSHR	GV V V A A						180	200	220	Human TSHR	TLKLYNNGFTSVQGYAFHGTLDVAVYLNKNKYLTVIDKDAFGGVYSGPSLLDVSQTSVTA				Dog TSHR	I H SA T Y						240	260	280	Human TSHR	LPSKGLKELKELIARNTNTLKLPLSLSLFLHLTRADLSYPHCCAFKNQKKIRGILESIM				Dog TSHR							300	320	340	Human TSHR	CHESHSQSLRQRKSVNALNSPLHQEYEEENLGDSTVGYREKSKFQDTHNNAHYTVFFEEQE				Dog TSHR	IR T G PD Y HA DN Q DS S			
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**Polypeptides having thyrotropin-receptor activity,
nucleic acid sequences coding for such
receptors and polypeptides,
and applications of these polypeptides**

The invention relates to polypeptides having thyrotropin-receptor activity, to nucleic acids coding for such polypeptides, to antibodies to these polypeptides and to the use of the polypeptides and antibodies in assay methods.

The literature references indicated by numbers in parentheses in this specification are listed in the form of a bibliography at the end of the description.

Pituitary glycoproteins (Luteinizing hormone, LH; follicle stimulating hormone, FSH ; and thyroid stimulating hormone or thyrotropin, TSH) form a family of closely related hormones.

The pituitary hormone thyrotropin (TSH) is the main physiological agent regulating the thyroid gland. It stimulates the function and the proliferation of thyrocytes and induces the expression of differentiation (1). Most of its effects are mediated by cyclic AMP (cAMP) (1). As the other pituitary and placental glycoprotein hormones (FSH, LH, CG), TSH is a heterodimer. All these hormones share an identical alpha subunit ; the beta subunit, despite sequence similarity, is specific for each (2). The activated TSH, FSH and LH-CG receptors stimulate adenylyl cyclase in their target cells via mechanisms mediated by the G protein Gs (3). In man, the TSH receptor may be the target of autoimmune reactions leading to hyper- or hypo-stimulation of the thyroid gland by autoantibodies in Grave's disease and in idiopathic myxoedema, respectively (4).

A prerequisite to studies of such diseases and to the elucidation of receptor structure and function is the availability of receptor preparations, particularly human, at a reasonable cost and in relative abundance.

To date, particulate membrane preparations and detergent-solubilised thyroid membranes, often of porcine or bovine origin (4) have been used in such studies. Human receptor preparations are not only costly but are also difficult to reproduce identically. Furthermore, the known preparations cannot be considered to be "purified" receptors; they are enriched with respect to their receptor content but do not allow purification of the receptor to a degree which would enable a partial sequence analysis, and hence its cloning. These receptor preparations have never allowed characterisation of the entity responsible for the TSH-binding activity.

Cloning and expression of the related LH-CG receptor has recently been achieved. A cDNA for the rat LH-CG receptor was isolated with use of a DNA probe generated in a polymerase chain reaction with oligonucleotide primers based on peptide sequences of purified receptor protein (15). Variants of the porcine LH-CG receptor were cloned by screening a λ gt11 library with cDNA probes isolated with monoclonal antibodies (16).

Attempts have been made to clone the TSH receptor (6) using a method which exploits the sequence similarity displayed by all known G-protein coupled receptors. A pair of oligonucleotide primers corresponding to transmembrane segments III and VI were used on cDNA from thyroid tissue under conditions allowing amplification of the primed sequences by the polymerase chain reaction. The method did not allow cloning of the TSH receptor but led instead to the

cloning of four new members of the G-protein coupled receptor family.

The difficulties encountered in purifying and in cloning the TSH receptor are thought to be due to its extra-ordinary low abundance even in thyroid cells.

The present inventors have succeeded in cloning the TSH receptor and variants thereof, firstly by applying the technique described in (6) but with different sets of primers, and with human genomic DNA as the template, rather than cDNA and secondly by use of a selected sequence amplified by this technique as a probe.

Certain aspects of the invention are illustrated in the figures 1 to 12. Figures illustrating amino-acid sequences use the one-letter abbreviation system.

Figure 1 is a sequence comparison of clone HGMP09 with a pannel of G-protein coupled receptors (6 and ref. therein). Only the sequence around transmembrane segment III of each receptor is shown in the one letter code. Residues conserved in HGMP09 and in more than 50 % of the other receptors are indicated by an asterisk. The "DRY" and "Asp113" residues (9) are indicated by ^.

Figure 2a shows the primary structure of the dog TSH receptor, as deduced from the nucleic acid sequence of dTSHr. The sequence was aligned (17) with full-length rat and pig LH-CG sequences (15, 16) and with HGMP09 partial sequence. Numbering is given from the first residue predicted in the mature polypeptide by von Heihne algorithm (11). Identical residues and conservative replacements in TSHr and LH-CGr are indicated by * and ., respectively. Sites for N glycosylation are underlined. Putative transmembrane segments are overlined. Lambda phages containing dTSHr inserts were subcloned in M13 and sequenced on both strands (Applied Biosystems model 370A)

using a combination of forced cloning and exonuclease III deletions (21).

Figure 2b is a dendrogram constructed from the sequences of G-protein coupled receptors. The CLUSTAL algorithm (17) was used to construct a dendrogram from the sequences of 22 receptors (6) and references therein) including rat and pig LH-CG receptors (16, 17), HGMP09 and the TSH receptor. For each receptor, a segment corresponding to the known sequence of HGMP09 (131 residues, extending from transmembrane segments II to V) was used for comparison by the program.

Figure 3a shows TSH induced morphological changes in Y1 cells microinjected with TSH receptor mRNA. Y1 cells were microinjected with recombinant TSH receptor mRNA (0.1 pl at 0.25 ug/ul) (right) or water (left) as described (13) and incubated in control medium (upper panel) or with TSH (0.1nM) (lower panel). RO 201724 and isobutylmethylxanthine (10^{-6} M each) were present in all incubations.

Figure 3b shows TSH induced cAMP accumulation in Xenopus oocytes microinjected with TSH receptor mRNA. Xenopus oocytes were handled as described (22) and injected with water (open symbols) or recombinant TSH receptor mRNA (13) (50 nl at 0.1 ug/ul) (filled symbols). After 3 days in control medium, batches of 35 oocytes were incubated for 90 min. in medium supplemented with various concentrations of TSH (circles), LH (squares) or FSH (triangles). cAMP was determined as described (14). RO 201724 and isobutylmethylxanthine (10^{-6} M each) were present in all incubations. Incubation of control oocytes in forskolin at 10^{-4} M resulted in doubling of the cAMP concentration (not shown).

Figure 4 illustrates the displacement of 125 I TSH receptors expressed in cos7 cells. Cos7 cells were

transfected with TSH receptor cDNA subcloned in pSVL (23). After 72 hours, cells were harvested and a membrane fraction was prepared (24). Membranes were similarly prepared from wild type cos7 cells and from dog thyrocytes in primary culture (20). Binding of ^{125}I TSH (TRAK Henning) was performed at 0°C for 120 min. in the presence of various concentrations of competitors (TSH-Armour, FSH and LH, UCB bioproducts). Bound radioactivity was separated by centrifugation and counted. Results are expressed as percent ^{125}I TSH bound by transfected cells in the absence of competitor (3,000 cpm) over non-specific binding (radioactivity bound in the presence of 100nM cold TSH, 800 cpm). Open and filled circles represent displacement by cold TSH from cos7 and thyrocyte membranes respectively. Open and filled squares represent displacement from cos7 by LH and FSH, respectively. Diamonds represent control cos7 cells in presence of various amounts of cold TSH.

Figure 5 shows the cDNA sequence coding for the dog TSH receptor, which was expressed in oocytes and culture cells.

Figure 6 is a shematic representation of the dog thyrotropin receptor, showing the 7 putative transmembrane segments and the large NH2 terminal extracellular domain (to the exclusion of the signal peptide). The amino-acids deleted in the variant form are indicated in black. The five putative glycosylation sites are shown.

Figure 7 shows the sequence alignment of the repeats constituting the extracellular domain of the thyrotropin receptor amino-acid sequence. The signal peptide, as determined by Von Heijne algorithm is represented in italic. The repeat missing in the molecular varian of the receptor is indicated by the leftward arrow.

Figure 8 shows the primary structure of the human TSH receptor as deduced from its cDNA sequence. The amino-acid sequence corresponds to the 2292 nucleotide open reading frame determined from the sequencing of two overlapping inserts in lamda gt11 clones (see examples). It is aligned for comparison with the dog TSH receptor sequence (only non conserved amino-acids are indicated). Numbering starts from the first residue of the mature polypeptide as determined by von Heijne algorithm [11]. Potential N-glycosylation sites are underlined and putative transmembrane segments are overlined.

Figure 9 shows the displacement by nonradioactive TSH of [¹²⁵I]TSH from human TSH receptors expressed in cos-7 cells. Results are expressed as percentage of the [¹²⁵I]-labelled TSH bound by transfected cells in the absence of competitor (1400 cpm) after correcting for nonspecific binding (radioactivity bound in the presence of 100 nM unlabelled TSH, 240 cpm).

Figure 10 represents the displacement by immunoglobulins of [¹²⁵I]TSH from human TSH receptor expressed in cos-7 cells. Results are expressed as described in the legend to fig. 9. Immoglobulins were prepared (see examples) from a normal individual (N), from patients with idiopathic myxoedema (IM1, IM2) or Graves' disease (GD1, GD2). The concentration of immunoglobulins in the assay is indicated. The ability of IM1 and IM2 (1.5 mg/ml) to inhibit TSH-stimulated cAMP production in a human thyrocyte assay was 100 % and 85 %, respectively. The thyroid stimulating activity of GD1 and GD2 (1.5 mg/ml) was equivalent to that of 10 mU/ml of TSH, respectively.

Figure 11 shows the primary structure of a TSH receptor according to the invention, in which a plurality

of letters at any one site indicates the presence of one of the given amino acid residues at that site.

Figure 12 illustrates the cDNA sequence of the cloned human TSH receptor.

The invention relates to polypeptides possessing thyrotropin receptor activity, characterised in that they comprise the amino-acid sequence shown in fig 11, or a fragment thereof, or an amino-acid sequence derived from this sequence by substitution or deletion of any of the amino-acid residues indicated in fig 11, or by insertion of additional amino-acid residues. Such derived sequences may show, for example, about 80 % homology with the sequence of figure 11. The polypeptides of the invention are in substantially pure form, and are preferably in a non-thyroid environment. By 'substantially pure form' is meant 'free of impurities' associated with detergent-solubilised thyroid membrane preparations.

By "TSH-receptor activity" is meant either TSH-binding properties or anti-TSH receptor antibody-binding properties or ability to activate adenylyl cyclase or phospholipase C via G proteins when exposed to TSH or anti-TSHr antibodies. These properties are easily verified by contacting the polypeptide with for example labelled TSH or labelled anti-TSHr antibodies or by monitoring the adenylyl cyclase activity of a membrane preparation containing the polypeptide. The polypeptides of the invention include the entire TSH receptor as identified by the inventors, and fragments or variants of this polypeptide as defined below. The entire TSH receptor is composed of a signal peptide (20 residues) followed by a large putative extracellular domain (398 residues) containing 5 sites for N-glycosylation, connected to a 346 residue COOH domain containing seven

putative transmembrane segments. The amino-acid sequence of the receptor is illustrated in fig. 11.

More particularly, the invention relates to a polypeptide characterised in that it comprises an amino-acid sequence represented by the following general formula:

$$[x]_n - [y]_m - [z]_p$$

wherein $n = 0$ or 1 ; $m = 0$ or 1 ; $p = 0$ or 1 ;

with the proviso that $n + m + p > 0$

and x , y and z are defined as follows (using the one-letter amino-acid symbol and wherein

a plurality of letters at any one site indicates the presence of one of the given amino-acid residues at that site,

$x = \text{MRPADLLQLVLLLDLPRDL},$

PP H A A S

$y =$ at least the minimum number of consecutive amino-acids of the following sequence necessary for the presentation of immunogenic properties:

GGMGCSSPPCECHQEEDFRVTCKDIQRIPSLPPSTQTLKLI
K P D H T F

ETHLRTIPSHAFSNLPNISRIYVSIDLTLQQLSHSFYNLSKVTHIEIRNTRNLTYIDPD
Q K R L A R M S S

ALKELPLLKFLGIFNTGLKMFDPDLTKVYSTDIFFILEITDNPYMTSIPVNAFQGLCNETL
GV V V A A

TLKLYNNGFTSVQGYAFNGTKLDAVYLNKNKYLTVIDKDAFGGVYSGPSLLDVSQTSVTA
I H SA T Y

LPSKGLEHLKELIARNTWTLKKLPLSLSLFLHLTRADLSYPSHCCAFKNQKKIRGILESIM

CNESSMQSLRQRKSVNALNSPLHQEYEENLGDSIVGYKEKSKFDTHNNAHYVFFEEQE
IR T G FD Y HA DN Q DS S

DEIIGFGQELKNPQEETLQAFDSHYDYTCGDSSEDMVCTPKSDEFNPCED
L V GN

and $z = [I - II - II_i - III - III_i - IV - V - VI - VII - VII_i]$

wherein the amino-acid sequences $I - II - II_i - III - III_i - IV - V - VI - VII - VII_i$ are independently present or absent and have the following meanings :

$I = \text{IMGYKFLRIVVWFVSL LALLGNV FVLLILLTSHYK}$

IV

or at least 12 consecutive amino-acid residues of this sequence;

$II = \text{LNVPRFLMCNLA FADFCMGMYLL LLIASVDLYTHSEYYNHA}$

T

I I

IH K Q H Y

or at least 12 consecutive amino-acid residues of this sequence;

$II_i =$

IDWQTGP GC

A

or at least 2 consecutive amino-acid residues of this sequence;

$III = \text{NTAGFFT VFA SELSVYTLTVITL}$

DA

or at least 22 consecutive amino-acid residues of this sequence;

$III_i =$

ERWYAITFAMRLD

HT H Q

or at least 2 consecutive amino-acid residues of this sequence;

10

IV = RKIRLRHACAIMVGGWVCCFLLALLPLVGISSYAKVSICL

C VQ YSV M IFA AA F IF M

A

or at least 12 consecutive amino-acid residues of this sequence;

V = PMDTETPLALAYIVFVLTLNIVAFVIVCCCYVKIYITVRN

IDS SQL VIL L VL I S

MSL V

or at least 12 consecutive amino-acid residues of this sequence;

VI = PQYNPGDKDTKIAKRMAVLIFTDFICMAPISFYALSAILNKPLIT

M

LM

or at least 12 consecutive amino-acid residues of this sequence;

VII = VSNSKILLVLFYPLNSCANPFLYAIFTKAFQRD

T

or at least 12 consecutive amino-acid residues of this sequence;

VII_i =

VFILLSKFGICKRQAQAYRGQRVPPKNSTDIQVQKVTHDMRQGLHNMEDVYELIENS

S AG I R S P Q E L

HLTPKKQGQISEEYMQTVL

N K N

or at least 12 consecutive amino-acid residues of this sequence;

it being understood that any of the above-specified amino-acids can be replaced or deleted, and that extra

amino-acid residues may be inserted provided the thyrotropin receptor activity is maintained.

The sequence represented by $[x]_n$ in the above general formula corresponds to the signal sequence of the TSH receptor. This part of the polypeptide naturally ensures the transport to the cell membrane of the adjoining $[y]$ and/or $[z]$ fragments, after its production in the cell. Clearly the signal sequence does not need to be present in the polypeptide in cases where transport to the membrane is not required (for example in in vitro translation of the mRNA encoding the polypeptide), or may be replaced by other signal sequences to facilitate production of the recombinant receptor in certain host cells.

The sequence represented by $[z]_p$ in the above general formula corresponds to the COOH domain of the entire polypeptide containing the seven putative transmembrane fragments I-VII, which show homology with the corresponding region of other G-protein coupled receptors. The polypeptides of the invention include, as indicated above, variants of the basic TSH receptor sequence lacking part or all of the transmembrane domain. It is thought that these types of variants may exist naturally as a result of an alternative splicing phenomenon. By homology with other, known G-protein coupled receptors, the seven putative transmembrane segments have tentatively been identified as shown in Fig. 11 (numbered I to VII). The variant polypeptides of the invention include polypeptides missing some or all of the fragments I - VII, as defined above, which definition includes the putative extracellular and intracellular "loops" occurring between the transmembrane segments (see fig. 6). The transmembrane segment(s) missing may therefore, for example, be a segment selected from

segments I to VII as indicated in fig. 11 or may be part of one of those segments, or may be a transmembrane segment in conjunction with its adjoining intracellular and/or extracellular loop.

It is also within the terms of the invention to replace some or all of the transmembrane domain by the transmembrane domain, or part of this domain, of a different receptor, thus giving rise to a hybrid receptor. This type of receptor is particularly interesting in cases where the extracellular part of the TSH receptor needs to be anchored in a cell membrane having characteristics which are different from, or even incompatible with, the transmembrane portion of the TSH receptor. It is also possible to use as the transmembrane domain in a hybrid receptor any amino-acid sequence exhibiting suitable anchoring properties. Such a sequence could be entirely synthetic or based on any transmembrane protein.

It is to be noted that the invention also embraces polypeptides having thyrotropin receptor activity which lack the entire transmembrane domain. In this case, the polypeptide corresponds to the extracellular domain of the naturally occurring receptor. This extracellular part of the receptor which is apparently responsible for ligand binding, is identified by the region [y] in the general formula. A polypeptide lacking the entire transmembrane domain is represented by the general formula [y]_m, where m = 1, the [z] part of the sequence being absent. This extracellular part of the receptor [y], is characterised by an imperfect repeat structure which can be aligned as shown in Fig 7. The polypeptides of the invention include variants in which one or more of these repeats is missing. It is however important that sufficient aminoacids be present to allow formation of

antibodies (monoclonal or polyclonal). Such immunogenic amino-acid sequences may comprise for example 5, 6, 7, 8 or 9 consecutive amino-acids of the "y" sequence defined above. The immunogenic nature of the fragments of the invention is tested by injection of the fragment in question into a laboratory animal, followed by investigation of the reactivity between any antibodies thus formed and the immunising fragment.

In particular, the invention encompasses polypeptides in which the second repeat (marked by an arrow in fig 7) is missing.

The invention also relates to nucleic acid sequences coding for the polypeptides of the invention as well as the corresponding complementary sequences. Examples of such sequences are those shown in figs. 5 and 12, and fragments of these sequences, as well as corresponding degenerate sequences. The nucleic acid fragments embraced by the invention normally have at least 8 nucleotides and have preferably at least 12 or preferably at least 16 nucleotides. Such fragments, or their complementary sequences can be used as primers in the amplification of segments of DNA using the polymerase chain reaction, for example in the production of cDNA coding for the polypeptides having thyrotropin receptor activity.

The nucleic acid sequences of the invention coding for the entire TSH receptor are in a genetic environment other than that found naturally in thyroid cells. For example, the genetic environment may be that of a Cos-7 cell, a CHO cell or Y1 cells.

The polypeptides of the invention can be produced in several different ways. For example, a host cell such as COS 7 cells, CHO cells, NIH3T3 cells, Xenopus oocytes or Y1 cells can be transformed by a vector containing a nucleic acid insert coding for the desired peptide, in

conjunction with all the necessary regulatory elements such as promoter, transcription termination signals etc, or can be microinjected with recombinant mRNA transcribed from appropriate vectors containing the receptor encoded sequence. Expression of the insert normally leads to the insertion of the recombinant polypeptide in the membrane of the cell used as host. In this way, the receptor polypeptide can be used in this form, the receptor thus being present in a non-thyroidal eukaryotic cellular environment, or in a solubilised membrane fragment form. The non-thyroid cells expressing the recombinant receptor exhibit a receptor density of upto ten times that observed in thyroid cells.

Furthermore, in the case where only a fragment of the polypeptide is required, the correspondingly shorter nucleic acid sequence can be used to transform a suitable host cell, for example, a DNA coding for the putative extracellular region only, or one or more repeats of the repetitive portion of this region. It is also within the terms of the invention to synthesise the polypeptide chemically, by successive assembly of the required amino-acid residues. In cases where larger fragments are desired, it is possible to synthesise first a series of smaller fragments and to ultimately assemble these fragments to form the larger fragment.

The invention also relates to antibodies, both polyclonal and monoclonal, to the thyrotropin-receptor polypeptides. The antibodies of the invention are preferably in a purified form, and may be of animal origin e.g. rabbit or mouse. As mentioned earlier, in man the TSH-receptor may be the target of auto-immune reactions giving rise to hyper- or hypo-stimulation of the thyroid gland by stimulating or blocking autoantibodies respectively. The antigenic nature of the

polypeptides of the invention, particularly the putative extracellular domain, permits the preparation of antibodies, which can be used in a variety of studies and assays. The TSH-receptor binds both TSH and anti-TSHr antibodies, thus it is possible in certain studies to replace TSH by anti-TSHr antibodies. The phenomenon of competition between labelled and unlabelled species is particularly useful in such assays. Use of specific fragments of the TSH receptor allows the preparation of antibodies against defined epitopes, and, by using a panel of such antibodies, allows further characterisation of the type of disorder present in auto-immune patients.

One such assay falling within the terms of the invention is a process for the quantitative detection of thyrotropine (TSH) or of anti-thyrotropine receptor antibodies (anti-TSHr) in a biological sample characterised in that a polypeptide according to the invention is contacted with the biological sample suspected of containing TSH or anti-TSHr antibodies and, either simultaneously or subsequently, contacted with labelled TSH, or with labelled anti-TSHr antibodies and the remaining, bound labelled TSH or bound labelled anti-TSHr antibodies after competition between the labelled and unlabelled species, is measured.

In this type of assay, the competition between the labelled TSH or labelled antibodies with the unlabelled TSH or antibodies present in the biological sample is measured as an indication of the concentration of that species in the sample.

Alternatively, instead of using competition between two like-species to measure TSH, it is also possible to use a receptor polypeptide to bind the TSH in the biological sample, and then after washing to add labelled anti-TSH antibodies which selectively detect the bound

TSH. This type of assay can also be carried out using immobilized or solubilised receptor polypeptide to bind the anti-TSHr-antibody in a biological sample, followed by detection of the bound antibody by labelled anti immunoglobulins or protein A or protein G or any other agent capable of recognizing an antibody.

Another means of assaying the TSH or anti-TSHr antibodies in a sample exploits the effect which the binding of these species with the TSH receptor has on the adenylyl cyclase activity of the cell bearing the receptor. Thus, this aspect of the inventions relates to a process for the quantitative detection of TSH or of anti-TSHr antibodies characterised by contacting intact cells operationally transformed by a nucleotide sequence, encoding a polypeptide of the invention or membrane preparations of such cells with the biological sample suspected of containing TSH or anti-TSHr antibodies and measuring in the intact cells or membranes the change in adenylyl cyclase activity, for example by measuring C-AMP generation or release.

The binding of TSH itself or of stimulating anti-TSHr antibodies to the receptor polypeptide leads to an increase in adenylyl cyclase activity, whereas the binding of blocking anti-TSHr antibodies leads to an inhibition of TSH-induced adenylyl cyclase stimulation. By comparing the adenylyl cyclase activity induced by exposure of the receptor polypeptide to TSH with that induced by antibodies in a sample, it is possible, according to the invention, to distinguish blocking antibodies from stimulating antibodies. In order to quantitatively determine blocking antibodies in a sample, the sample is contacted with the receptor polypeptides either at the same time as with TSH, or before contacting with TSH. In this way the adenylyl cyclase stimulating

effect of TSH on the receptor is blocked by the blocking antibodies and is quantified to indicate the concentration of blocking antibodies present in the sample. Such measurements can be carried out in intact cells bearing the TSH receptors of the invention, or in membrane preparations of such cells. Other effector systems which can be used in this type of detection are, for example, activities of phospholipase C, protein tyrosine kinase, phospholipase A2 etc.

The labels used in the assays of the invention are those conventionally used in the art, for example, radioactive labelling, enzymatic labelling, labelled anti-immunoglobulins, protein A, protein G, depending upon the type of assay.

Another aspect of the invention relates to a process for the quantitative detection of fragments of TSH receptor in a biological fluid. Such fragments may be found circulating in patients suffering from thyroid disorders. This aspect of the invention involves contacting the sample with antibodies according to the invention which have previously been labelled, if necessary, and determining the binding, if any, in the sample by any method involving separation of bound labelled antibody from unbound labelled antibody or by competition between the said fragments and a polypeptide according to the invention. In this latter case it is necessary to label the receptor polypeptide, for example with ^{125}I .

The antibodies of the invention may also be used in the immunohistochemical detection of TSH receptors, for example in endocrinological investigations or in anatomopathology. In this type of process, the antibodies are again labelled to permit their detection.

The polypeptides of the invention may also be used in the purification of stimulating or blocking antibodies to TSHr and of TSH by contacting the polypeptide with a source of TSH or anti-TSHr antibodies, separating the bound and free fractions and finally dissociating the receptor-bound entity. If necessary, further successive purification steps known per se may be added. Such a purification process is facilitated by the immobilisation of the receptor polypeptide, for example in a column or any other solid support.

The invention also embraces kits suitable for the detection of TSH or anti-TSHr antibodies. Such kits are characterised in that they contain:

- a) a polypeptide according to the invention and defined above, said polypeptide having thyrotropin receptor activity and being either in an immobilised or solubilised form ;
- b) at least one of the following reagents:
 - i) labelled TSH
 - ii) labelled anti-TSHr antibodies
 - iii) reagents necessary for the measurement of adenylyl cyclase activity.

For example, a kit for effecting the detection of autoantibodies directed against the TSH receptor by competition would include the polypeptide of the invention, in immobilised or solubilised form, with labelled TSH or unlabelled TSH in combination with agents permitting the TSH to be labelled. Alternatively, such a kit might include antibodies to the TSH receptor and means of labelling them, instead of the TSH.

The invention will be illustrated by the following examples:

Examples

I - Cloning of dog TSHr

a) Identification of HGMP09

As most G protein-coupled receptor genes do not contain introns in their coding sequence, a similar strategy to that previously described (6) was used, but using different sets of degenerated primers and with human genomic DNA as starting material. Eleven clones displaying sequence similarity with G-protein coupled receptors were thus obtained (7). Out of these, one clone (HGMP09) which was amplified with primers corresponding to transmembrane segments II and VII, presented sequence characteristics suggesting that it belonged to a distinct subfamily of receptors.

The primers used in this amplification were:

5' TAGATCTAGACCTGGCGITTGCCGATCT 3'
 T T C GC T CA
 G

and 5' ACTTAAGCTTGCACTAGCCCAIAGGATT 3'
 A AAAG G G

a plurality of nucleotides at any one site indicating the presence of one of the given nucleotides at that site.

A dendrogram constructed from the alignment shown in fig. 1 demonstrated that it is equally distant from all receptors cloned to date (7); in particular, it does not contain the canonical Asp Arg Tyr (DRY) tripeptide close to transmembrane segment III⁽⁸⁾ and lacks the Asp residue implicated in the binding of charged amines in adrenergic (Asp113), muscarinic, dopaminergic and serotonergic receptors (9).

b) Identification of dog TSHr

In the frame of a systematic screening for the expression of the new receptors in thyroid tissue, HGMP09 was used as a probe both in Northern blotting experiments with thyroid and non-thyroid tissues, and in screening of a dog thyroid cDNA library. HGMP09 did not hybridize to

thyroid mRNA but identified a major 2.6 kb transcript in the ovary and the testis. However, under moderate conditions of stringency it hybridized to one out of 50,000 thyroid cDNA clones suggesting cross-hybridization with a relatively abundant putative receptor of the thyroid. From these characteristics, it was hypothesized that HGMP09 encoded a receptor fragment, distinct from the TSH receptor, but with sequence characteristics expected from close relatives like LH or FSH receptors. A full-length cross-hybridizing clone (dTSHr) was isolated and used as a probe in Northern blots of ten different dog tissues. It hybridized to a 4.9 kb transcript present only in the thyroid gland and in cultured thyrocytes. Interestingly, the signal was much stronger in cultured thyrocytes exposed for several days to the cAMP agonist forskolin than in thyroid tissue. This is a characteristic one would expect from the TSH receptor whose expression is known to be up-regulated by cAMP agonists in cultured cells (10). A 4,417 bp cDNA clone was sequenced completely. It contains an open reading frame of 764 aminoacids beginning with a 20 residue signal peptide, as predicted by Von Heijne algorithm (11) (fig.2a). Comparison to known G-protein coupled receptors (see hereunder and fig. 2b) and hydropathy profile analysis (not shown) demonstrated a 346 residue C-terminal structure with seven putative transmembrane domains preceded by 398 aminoacids constituting a large putative extracellular domain.

c) Expression of dog TSHr

The encoded polypeptide was unambiguously identified as the TSH receptor by expression of the cDNA in a variety of systems. Microinjection of recombinant mRNA in adrenocortical Y1 cells and in *Xenopus* oocytes conferred a TSH responsive phenotype to both systems. Y1 cells

responded to TSH by a characteristic morphological change which is triggered by elevation of cAMP in the cytoplasm (12,13). *Xenopus* oocytes (fig. 3) displayed a dose-dependant increase in cAMP which was specific for stimulation by TSH and corresponded to the expected sensitivity of the dog receptor to bovine TSH (half-maximal effect around 0.3 nM) (14). Transient expression of the receptor cDNA was obtained in Cos7 cells (fig 4). Specific binding of ^{125}I TSH to membranes was observed only in transfected cells. The displacement curve of the label by TSH presented characteristics very similar to that obtained with membranes from dog thyrocytes (half-maximal displacement at 0.4 nM and 0.16 nM for cos cells and thyrocytes, respectively) (fig. 4c). The slight rightward shift of the displacement curve obtained with Cos7 cell membranes may reflect the higher amount of receptors in this system.

The cDNA coding for the dog TSH receptor was sequenced completely. The sequence is given in fig. 5.

d) Comparison of TSHr with LH-CGr

Comparison of the TSH receptor with the LH-CG receptor cloned recently (15, 16) reveals interesting common characteristics which make them members of a new subfamily of G-protein coupled receptors. They both display a long aminoterminal extension containing multiple sites for N glycosylation (five in the TSH receptor). The TSH receptor has an extra 52 residue insert close to the junction between the putative extracellular domain and the first transmembrane segment (fig. 2a). The overall sequence similarity between the extracellular domains of the TSH and LH-CG receptors is 45% (fig 2a). The similarity between a segment of soybean lectin and the rat LH receptor (15) is not conserved in the TSH receptor, which suggests that it may be

fortuitous. The C-terminal half of the TSH receptor containing the transmembrane segments is 70% similar to both the pig and rat LH receptors (fig. 2a). The homology is particularly impressive in the transmembrane segments themselves, where stretches of up to 24 identical residues are observed in a row (transmembrane region III). Also, the carboxyl terminal region of the third putative intracellular loop, which is particularly short in TSH and LH receptors and which has been implicated in the interaction with $G_{\alpha s}$ (8, 9), is identical in both receptor types. This pattern of similarities gives support to the view that the extracellular domain would be involved in the recognition of the ligands (4), while the membrane-inserted domain would be responsible for the activation of $G_{\alpha s}$ (15, 16). Together, the TSH and LH-CG receptors, and HGMP09 (there is strong preliminary evidence that HGMP09 could actually be the FSH receptor (7)) constitute clearly a distinct subfamily of G-protein coupled receptors. A sequence similarity dendrogram (17) including most of the G-protein coupled receptors cloned to date demonstrates both their close relationships and their distance from the bulk of the other receptors (fig. 2b). The complete sequence of the FSH receptor will reveal whether the known ability of LH-CG to stimulate the TSH receptor (18) is reflected by a higher sequence similarity of the extracellular domains of LH and TSH receptors.

e) Identification of a dog TSHr variant

Screening of the dog thyroid cDNA library (30) with the HGMP09 clone (thought to be part of the FSH receptor), gave rise to 16 positive clones out of the 840,000 screened plaques. Hybridization was carried out at 42°C in 35% formamide and the filters were washed at 65°C in 2 x SSC, 0.1% SDS before autoradiography. 12

clones were purified to homogeneity and analyzed by EcoRI digestion. Three clones (dTSHR1, dTSHR2 and dTSHR3) were subcloned in M13mp18 and pBS vectors. dTSHR1 and dTSHR2 consisted of two EcoRI fragments of respectively 2800 and 1500 bp. dTSHR3 was shorter, and consisted of 2200 and 1500 bp EcoRI fragments. Restriction analysis of the 2800 bp fragments of dTSHR1 and dTSHR2 revealed slight differences in the restriction map, the main discordance being the presence of a PstI restriction site in dTSHR1 and its absence in dTSHR2. dTSHR1 was sequenced completely and revealed an open reading frame of 764 codons which was identified as the thyrotropin receptor by expression of the cDNA in oocytes and cell cultures (see example I(b) + fig 5). dTSHR3 was shown by sequencing to be completely colinear with dTSHR1 but this clone lacked 600 bp at its 5' end. Because of the difference in the restriction map of dTSHR1 and dTSHR2, this latter clone was also sequenced on both strands.

The sequence revealed a number of mutations when compared with the dTSHR1 clone. A total of 5 mutations, including two single base substitutions, one single base deletion, one single base insertion and one 5 base insertion were found scattered in the 2060 bp long 3' untranslated region (not shown). However, the main difference between dTSHR2 and dTSHR1 was located in the coding region, and consisted in a 75 bp deletion located 240 bp after the start codon. The corresponding 25 amino-acids deletion in the protein itself is located in the long NH2 terminal extracellular domain which is characteristic of the TSH receptor (25) and its recently cloned close relative, the LH receptor (15, 16) (fig. 6). As in the LH receptor, the NH2 terminal part of the thyrotropin receptor is characterized by an imperfect repeat structure that can be aligned as indicated in

fig. 7. These repeats are similar in structure to the leucine-rich repeats found in the various proteins comprising the family of leucine-rich glycoproteins (26, 15), and references therein). The deletion in the dTSHR2 clone corresponds exactly to one of these repeats, in a region of the protein where the repeat length is regular and their alignment unambiguous. The existence of such variant reinforces considerably the significance of this repeated structure and sets up interesting questions concerning its functional meaning and the structure of the chromosomal gene.

The extracellular domains of TSH and LH receptors are apparently responsible for the ligand binding (4). The deleted repeat also contains one of the 5 consensus sequences for N-glycosylation. Glycosylation of the TSH receptor could be important for ligand binding or signal transduction. If, and to what extent, the lack of this repeat influences the binding capabilities and/or the function of the receptor variant, is not yet known. Comparison of cell lines expressing this variant with the presently available stable transfectants expressing the full size receptor should partially answer this question. The functional analysis of other in-vitro generated mutants of the TSH receptor will complete the study.

The deletion of a full repeat gives also some insight on the structure of the TSH receptor gene. It is highly probable that the repeat unit corresponds to a complete exon, and it is therefore possible that all repeats would be separated by introns. It is interesting to note that most of the genes coding for G-protein coupled receptors are completely devoid of intronic structures. The functional or evolutionary significance of this observation is not known, but a highly fragmented exonic structure of the glycoprotein hormone receptor

genes would be in clear contrast to the rest of the receptor family.

II - Cloning of the human TSHr

A human lambda gt11 cDNA library (29) was screened with a fragment of the dog TSHr (25). Out of the 218 clones scored as positive (1/6000), 24 were plaque-purified to homogeneity and the size of the inserts was determined. Two clones which harbored inserts of 2370 bp and 3050 bp, respectively, were subcloned as overlapping fragments in M13 derivatives and sequenced (fig 12). A total of 4272 bp were determined in which a 2292 bp open reading frame was identified. When translated into protein, the coding sequence showed an overall 90.3 % similarity with the dog TSHr (Fig. 8) [1]. It displayed all the characteristics described recently for the subfamily of G protein-coupled receptors binding glycoprotein hormones (25, 15, 16); a signal peptide (20 residues) followed by a large putative extracellular domain (398 residues) containing 5 sites for N-glycosylation, connected to a 346 residue carboxyl-terminal domain containing seven putative transmembrane segments (fig. 8). It has been suggested that the amino-terminal domain, which is not found in other G protein-coupled receptors, might correspond to the region involved in the binding of the bulky pituitary and placental glycoprotein hormones (25, 15, 16).

Variants of the hTSHr

When probed with the putative human TSHr, a Northern blot of RNA from human placenta, testis and thyroid revealed two major 4.6 and 4.4 kb thyroid-specific transcripts. Minor thyroid-specific RNA species of smaller size were also observed. Although the former could simply correspond to multiple polyadenylation sites in the 3' region of the gene, this situation is reminiscent of what

has been observed for the porcine LH-CG receptor. In this case, multiple transcripts were found to correspond to variants of the receptor cDNA lacking the potential to encode the membrane spanning segments (16). Whether this observation with important implications on receptor function and regulation also applies to the human TSHr will await sequencing of additional clones from the cDNA library.

Expression of hTSHr

To provide definite proof that the clones isolated encoded a human TSH receptor, the coding sequence was inserted in the SV40-based vector pSVL, and the resulting construct transfected in Cos-7 cells (24). Membranes prepared from transfected cells demonstrated specific binding of [125 I]TSH (fig. 9). The unlabelled competitor TSH was bovine. The characteristics of the displacement curve with unlabelled TSH were similar to those observed with the dog TSHr assayed under similar conditions (half maximal displacement around 0.5 nM) (25).

From the sequence similarity with dog TSHr, the tissue specific expression of the corresponding transcripts and the binding studies on membranes from transfected COS-7 cells, it was concluded that a bona fide human TSHr has been cloned.

Antibodies to hTSHr

To investigate the relevance of the cloned human TSHr to thyroid autoimmunity, competition was tested between [125 I]TSH and immunoglobulins prepared from patients, for binding to the recombinant receptor expressed in Cos-7 cells (fig 10). Immunoglobulins were prepared from the serum of patients or normal individuals by ammonium sulphate precipitation. They were dissolved in water and dialysed extensively against Dulbecco's modified Eagle medium. While immunoglobulins from normal

individuals did not displace [125 I]TSH, samples from two patients with idiopathic myxoedema clearly did, in a dose-dependant manner. The steep dose-response which was observed suggests that immunoglobulins from these patients present a very high affinity for the recombinant receptor. When samples from two patients with Graves' disease having high levels of thyroid stimulating immunoglobulins in the circulation were tested, one of them showed limited ability to displace labelled TSH under the conditions of the assay (fig.10). The difference in the potency of these two categories of immunoglobulins to displace TSH from the receptor expressed in Cos-7 cells may reflect differences in their affinity for a common antigen. Alternatively, despite previous studies suggesting that both stimulating and blocking antibodies would bind to the same part of the TSHr (26, 27), it may correspond to more basic differences in the actual nature of their respective antigenic targets. Studies where binding activity of a larger collection of immunoglobulins are compared to their ability to activate adenylate cyclase in permanently transfected cells will help to clarify this point.

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were marked on the bottom of the dishes and all cells in these areas were microinjected with mRNA at 0.25 ug/ul in water. mRNA was synthesized from TSH receptor cDNA subcloned in pSP64 (Promega). After 30 min; TSH was added and the cells were photographed 120 min. later. The morphological changes (stable for 20 hours) were observed with TSH concentrations down to 0.1 nM. FSH, LH and hCG were ineffective (not shown).

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Claims

1. Polypeptide possessing thyrotropin receptor activity, characterised in that it comprises the amino-acid sequence shown in fig 11, or a fragment thereof, or an amino-acid sequence derived from this sequence by substitution or deletion of any of the amino-acid residues indicated in fig 11, or by insertion of additional amino-acid residues.

2. Polypeptide according to claim 1, characterised in that it comprises an amino-acid sequence represented by the following general formula:

$$[X]_n - [Y]_m - [Z]_p$$

wherein $n = 0$ or 1 ; $m = 0$ or 1 ; $p = 0$ or 1 ;

with the proviso that $n + m + p > 0$

and x , y and z are defined as follows (using the one-letter amino-acid symbol and wherein a plurality of letters at any one site indicates the presence of one of the given amino-acid residues at that site):

$x =$ MRPADLLQLVLLLDLPRDL,

PP H A A S

$y =$ at least the minimum number of consecutive amino-acids of the following sequence necessary for the presentation of immunogenic properties:

GGMGCSSPPCECHQEEDFRVTCKDIQRIPSLPPSTQTLKLI
K P D H T F

ETHLRTIPSHAFSNLPNISRIYVSIDLTLQQLSHSFYNLSKVTHIEIRNTRNLTYIDPD
Q K R L A R M S S

ALKELPLLKFLGIFNTGLKMFDPDLTKVYSTDIFFILEITDNPYMTSIPVNAFQGLCNETL
GV V V A A

TLKLYNNGFTSVQGYAFNGTKLDAVYLNKNKYLTVIDKDAFGGVYSGPSILLDVSQTSVTA
I H SA T Y

LPSKGLEHLKELIARNTWTLKKLPLSLSFLHLTRADLSYP SHCCAFKNQKKIRGILESIM

CNESSMQSLRQRKSVNALNSPLHQEYEENLGDSIVGYKEKSKFQDTHNNAHYVVFEEQE
IR T G FD Y HA DN Q DS S

DEIIGFGQELKNPQEETLQAFDSHYDYTI CGDSEDVCTPKSDEFNCPED
L V GN

and $z = [I - II - II_i - III - III_i - IV - V - VI - VII - VII_i]$

wherein the amino-acid sequences I - II - II₁ - III - III₁ - IV - V - VI - VII - VII₁ are independently present or absent and have the following meanings:

I = IMGYKFLRIVVWFVSLALLGNV FVLLILLTSHYK
IV

or at least 12 consecutive amino-acid residues of this sequence;

II = LNVPRFLMCNLAFADFCMGMYLLLIASVDLYTHSEYYNHA
T I I I H K Q H Y

or at least 12 consecutive amino-acid residues of this sequence;

II, =
IDWQTGPGC
A

or at least 2 consecutive amino-acid residues of this sequence;

III = NTAGFFT VFAS ELSVYTLTVITL
DA

33

or at least 22 consecutive amino-acid residues of this sequence;

III₁ =
ERWYAITFAMRLD
HT H Q

or at least 2 consecutive amino-acid residues of this sequence;

IV = RKIRLRHACAIMVGGWCCFLLALLPLVGISSYAKVSICL
C VQ YSV M IFA AA F IF M
A

or at least 12 consecutive amino-acid residues of this sequence;

V = PMDTETPLALAYIVFVLTNLNIVAFVIVCCCYVKIYITVRN
IDS SQL VIL L VL I S
MSL V

or at least 12 consecutive amino-acid residues of this sequence;

VI = PQYNPGDKDTKIAKRMAVLIFTDFICMAPISFYALSAILNKPLIT
M LM

or at least 12 consecutive amino-acid residues of this sequence;

VII = VSNSKILLVLFYPLNSCANPFLYAIFTKAFQRD
T

or at least 12 consecutive amino-acid residues of this sequence;

34

VII₁ =

VFILLSKFGICKRQAQAYRGQRVPPKNSTDIQVQKVTHDMRQGLHNMEDVYELIENS

S AG I R S P Q E L

HLTPKKQGQISEEYMQTVL

N K N

or at least 12 consecutive amino-acid residues of this sequence;

it being understood that any of the above-specified amino-acids can be replaced or deleted, and that extra amino-acid residues may be inserted provided the thyrotropin receptor activity is maintained.

3. Polypeptide according to claim 1, characterised in that "y" is composed of at least one of the following sub-sequences : y₁ to y₁₃:

Y1 : GMGCSSPPCECHQEEDFRVTCKDIQRIPSLPPSTQT

K P D H T

Y2 : LKLIETHLRTIPSHAFSNLPNISR

F Q K R

Y3 : IYVSIDLTLOQLESHSFYNLSKVTH

L A R M

Y4 : IEIRNTRNLTYIDPDALKELPLLKF

S S

Y5 : LGIFNTGLKMFDPDLTKVYSTDIFFI

GV V V

Y6 : LEITDNPYMTSIPVNAFQGLCNETL

A A

Y7 : TLKLYNNGFTSVQGYAFNGTKLDAV

I H

35

Y8 : YLNKNKYLTVIDKDAFGGVYSGPS

SA

T

Y9 : LLDVSQTSVTALPSKGLEHLKELIA

Y

Y10 : RNTWTLKKLPLSLFLHLTRADL

Y11 : SYPSHCCAFKNQKKIRGILESLMCN

Y12 : ESSMQSLRQRKSVNALNSPLHQEYE

IR

T G FD

Y13:

ENLGDSIVGYKEKSKFQDTHNNAHYVFFEEQEDEIIGFGQELKNPQEETLQAFDSH

Y

HA

DN Q

DS S

L

YDYTICGDSEDMVCTPKSDEFNPCED

V GN

4. Polypeptide according to any of claims 1 to 3, in which part of the polypeptide is heterologous to the TSH receptor, for example the part forming the transmembrane domain, the polypeptide thus being a hybrid polypeptide.

5. Polypeptide according to claim 1, characterised by the sequence shown in fig. 11.

6. Nucleotide sequence coding for the polypeptide according to claims 1 to 5, and fragments of such sequences having at least 8 nucleotides, preferably at least 12 nucleotides, and most preferably at least 16 nucleotides, and nucleotide sequences which are complementary to said sequence or fragment.

7. Nucleotide sequence according to claim 5 characterised in that the sequence is a DNA sequence having the sequence shown in fig. 5 or fig. 12, or fragments of this sequence having at least 8 nucleotides,

preferably at least 12 nucleotides, and most preferably at least 16 nucleotides, or sequence complementary to said sequence or fragment.

8. Process for the preparation of a polypeptide according to any one of claims 1 to 5 by the expression of a nucleic acid coding for the polypeptide in a host cell transformed by a vector in which the said nucleic acid has been operationally inserted.

9. Process for the quantitative detection of thyrotropine (TSH) or of anti-thyrotropine receptor antibodies (anti-TSHr) in a biological sample characterised in that a polypeptide according to any one of claims 1-5 is contacted with the biological sample suspected of containing TSH or anti-TSHr antibodies, and either simultaneously or subsequently is contacted with labelled TSH, or with labelled anti-TSHr antibodies and the remaining, bound labelled TSH or bound labelled anti-TSHr antibodies, after competition between the labelled and unlabelled species, is measured.

10. Process for the quantitative detection of TSH or of anti-TSHr antibodies characterised by contacting intact cells operationally transformed by a nucleotide sequence, according to claim 5 or 6 or membrane preparations of such cells with the biological sample suspected of containing TSH or anti-TSHr antibodies and measuring in the intact cells or membranes the change in adenylyl cyclase activity, for example by measuring C-AMP generation or release.

11. Process according to claim 10 characterised in that the intact cells expressing the receptor polypeptide or the membranes of such cells are contacted with the biological sample and, either simultaneously or subsequently with TSH, thereby allowing any inhibition of the adenylyl cyclase activating effect of TSH by

"blocking" anti-TSHr antibodies present in the biological sample to be detected.

12. Polyclonal or monoclonal antibodies to the receptor polypeptide of claims 1 to 5.

13. Process for the purification of TSH or of stimulating and blocking antibodies to TSHr by contacting a polypeptide according to any one of claims 1 to 5 with a source of TSH or anti TSHr antibodies, followed by the separation of bound and free entities, and dissociation of the receptor-bound TSH or antibodies.

14. Process for the quantitative detection of fragments of TSHr in a biological sample by contacting the sample with antibodies according to claim 12, which have previously been labelled, if necessary, and determining the binding, if any, in the sample, by any method involving separation of the bound labelled antibody from unbound labelled antibody, or by competition between the said fragments and a polypeptide according to any one of claims 1 to 5 for the said antibodies, said polypeptide being labelled for example by ^{125}I .

15. Use of the antibodies according to claim 12 in the immunohistochemical detection of TSH receptor in a biological sample.

16. Kit for the detection of TSH or anti-TSHr antibodies characterised in that it contains:

- a) Polypeptide according to any one of claims 1 to 5, having thyrotropin receptor activity and being either in an immobilised or solubilised form ;
- b) at least one of the following reagents:
 - i) labelled TSH
 - ii) labelled anti-TSHr antibodies
 - iii) reagents necessary for the measurement of adenylyl cyclase activity.

17. Kit according to claim 16, characterised in that the polypeptide is present in the form of intact cells previously operationally transformed by a nucleotide sequence coding for said polypeptide and consequently bearing said polypeptide in its membrane, or in the form of solubilized membranes of such cells.

FIGURE 1

3rd transmembrane segment	
HGMP09	CDA AGFFTTFASELSVYTLTAITL ERWHTITHAMQLDCKVQLRHAASVMVMG-WIFAF
M2HUM	CDL WLALDYVVSNASVMNLLIISF DRYFCVTKPLTYPVKRTTKMAGMMIAAA-WVLSF
M3HUM	CDL WLALDYVVSNASVMNLLIISF DRYFCVTKPLTYPARRTTKMAGLMAAA-WVLSF
M1HUM	CDL WLALDYVASNASVMNLLIISF DRYFSVTRPLSYRAKRTTPRRAALMIGLA-WLVSF
M4HUM	CDL WLAIDYVASNASVMNLLVVSF DRYFSITRPLTYRAKRTTKRAGVMIGLA-WVVSF
A1ADRHAM	CDI WAAVDVLCCTASILSLCAISI DRYIGVRSYSLQYPTLVTRRKAILALLSV-WVLSF
D2R	CDI FVTLDVMMCTASILNLCAISI DRYTAVAMPMLYNTRYSSKRRVTVMIAIVWVLSF
B1ADRHUM	CEL WTSVDVLCVTASIELTLCVIAL DRYLAITSFQRYQSLLTRARARGLVCTV-WAISA
B2ADRHUM	CEF WTSIDVLCVTASIELTLCVIAV DRYFAITSFQYQSLLTKNKARVIILMV-WIVSG
BADRHAM	CEF WTSIDVLCVTASIELTLCVIAV DRYIAITSFQYQSLLTKNKARMIILMV-WIVSG
RDC4	CDI WLSSDITCCTASILHLCVIAL DRYWAITDALEYSKRRTAGRAAVMIATV-WVISI
A2ADRHUM	CEI YLALDVLCCTSSIVHLCAISL DRYWSITQAI EYNLKRTRRRRIKAIITC-WVISA
G215HT1A	CDL FIALDVLCCTSSIVHLCAIAL DRYWAITDPI DYVNKRTP-RPRALISLT-WLIGF
5HT1CRAT	CPV WISLDVLCCTSSIVHLCAISL DRYVAIRNPIEHSRFRNSRTKAIMKIAIV-WAISI
5HT2R	CAI WIYLDVLCCTSSIVHLCAISL DRYVAIQNP IHSRFRNSRTKAFKIIAV-WTISV
RDC8	CLF FACFVLVLTQSSIFSLAIAI DRYIAIRIPLRYNGLVTGTRAKGIIAVC-WVLSF
RDC7	CLM VACPVLILTQSSIFSLAIAV DRYLRVKIPLRYKTVTPRRAAAVAIAGC-WVLSF
SKRBOV	CYF QNLFPITAMFVSIYSMTAIAA DRYMAIVHPFQP--RLSAPGTRAVIAGI-WLVAL
RDC1	CKI THLIFSINLFGSIFFLTCMSV DRYLSITYFASTSSRRKKVVRRAVCVLV-WLLAF

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-20.      1.
DOGTSH : MRPPPLHLALLALPRS-----LGGKGCSPPCÉCHQEDDFRVT
RATHCG : MGRRVPALRQLLVALLKPSQLQSRELSGSRCPÉ-PCDCAPDGAL---
PIGCHG : MRRRSLALR--LLLALLPPPLPQT--LLGAPCPE-PCSCRDPGAL---
          * **.* ** * * * ** ** * . . .
          .

50.
DOGTSH : CKDIHRIPTLPSTQTLKFIE1TQLKTIPSR2AFSNLPNISRIYLSIDATLQ
RATHCG : -----RCPGPRAGLARLSLT3YLPVKVIP4PSQAFRLNEVV5KIEISQSDSLE
PIGCHG : -----RCPGPRAGLSRLSLTYLP6IKVIP7PSQAFRLNEVV8KIEISQSDSLE
          * * * * * . * ** ** * * . . . * * * .
          .

100.
DOGTSH : RLESHFYNL1SKMTHIEIRNTRSLTSIDPDALKELPLLKFLGIFNTGLGV
RATHCG : RIEANAFDNLNLSELLIQNTKNLLYIEPGAF2TNLPRLKYL3SI4CNTGIR5T
PIGCHG : KIEANAFDNLNLSEILLIQNTKNLVYIEPGAF6TNLPRLKYL7SI8CNTGIRK
          . * * * . . * * * . * * * . * * * * * * .
          .

150.
DOGTSH : FPDVTKVYSTDVFFILEITDNPYMASIPANAFQGLCNET1ITLKL2YNNGFT
RATHCG : LPDVTKISSSEFNFILEICDNLHIT3TIPGNAFQGMN4NE5VTLKL6YGN7GFE
PIGCHG : LPDVTKIFSSSEFNFILEICDNLHIT8TVPANAFQGMN9NE10ITLKL11YGN12GFE
          . * * * * . * * * * * . * * * * * . * * * * * * .
          .

```

FIGURE 2a

200.
 DOGTSH : SIQCHAFNGTKLDAVYLNKNKYLSAIDKDAFGGVYSGPTLLDVSYTSVTA
 RATHCG : EVQSHAFNGTTLISLELKENIYLEKMHSGAFQCAT-GPSILDISSTKLQA
 PIGHCG : EIQSHAFNGTTLISLELKENAHLKKMHNDAFRGAR-GPSILDISSTKLQA
 . * * * * * . * * * * * . * * * * * . * * * * * . *

250.
 DOGTSH : LPSKGLEHLKELIARNTWTLLKKPLSLSLFLHLTRADLSYPSSHCCAFKNQK
 RATHCG : LPSHGLESIQTLIALSSYSLSKTLPSKEKFTSLLVATLTYPSSHCCAFRNLP
 PIGHCG : LPSYGLESIQTLIATSSYSLSKTLPSREKFTNLLDADTLTYPSSHCCAFRNLP
 *** * * * * . * * * * * . * * * * * . * * * * * . *

300.
 DOGTSH : -KIRGILESMLCNESIRSRLQRKSVN-TLNGPFDQEYELGDSHAGYK
 RATHCG : KKEONFSFSIFENFSKQCESTVRKADNETLYSAIFEENELSGWD-----
 PIGHCG : TKEONFSFSIFKNFSKQCESTARRPNNETLYSAIFAESELSWD-----
 * * * * * . * * * * * . * * * * * . * * * * * . *

350.
 DOGTSH : DNSQFQDTSNSHYVVFEEQEDEILGFGQELKNPQEETLQAFDSDYD
 RATHCG : -----YDYG
 PIGHCG : -----YDYG
 -----**

FIGURE 2a - SUITE 1

V
 DOGTSH : L N I V A F I I V C S C Y V K I Y I T V R N P Q Y N P G D K D T K I A K R M A V L I F T D F M C M A VI
 RATHCG : L N V V A F V V I C A C Y I R I Y F A V Q N P E L T A P N K D T K I A K K M A I L I F T D F T C M A
 P I G H C G : L N V V A F I I I C A C Y I K I Y F A V Q N P E L M A T N K D T K I A K K M A V L I F T D F T C M A
 H G M P 0 9 : L N V L A F
 **.* **.* **.* **.* **.* **.* **.* **.* **.* **.*

VI
 DOGTSH : P I S F Y A L S A L M N K P L I T V T N S K I L L V L F Y P P L N S C A N P F L Y A I F T K A F Q R D
 RATHCG : P I S F F A I S A A F K V P L I T V T N S K I L L V L F Y P P V N S C A N P F L Y A I F T K A F Q R D
 P I G H C G : P I S F F A I S A A L K V P L I T V T N S K V L L V L F Y P V N S C A N P F L Y A I F T K A F R R D
 H G M P 0 9 :
 ***.* **.* **.* **.* **.* **.* **.* **.* **.* **.*

700.
 DOGTSH : V F I L L S K F G I C K R Q A Q A Y R G Q R V S P - - - - K N S A G I Q I Q K V T R D M R Q S L P
 RATHCG : F L L L S R F G C C K R R A E L Y R R K E F S A Y T S N C K N G F P G A S K P S Q A T L K L S T V
 P I G H C G : F F L L L S K S G C C K H Q A E L Y R R K D F S A Y - - - C K N G F T G S N K P S R S T L K L T T L
 ..***.* **.* **.* **.* **.* **.* **.* **.* **.* **.*

DOGTSH : N M Q D E Y E L L E N S H L T P N K Q G Q I S K E Y N Q T V L
 RATHCG : H C Q Q P I P P R A L T H
 P I G H C G : Q C Q Y S T V M D K T C Y K D C
 *

FIGURE 2a - SUITE 3

FIGURE 2b

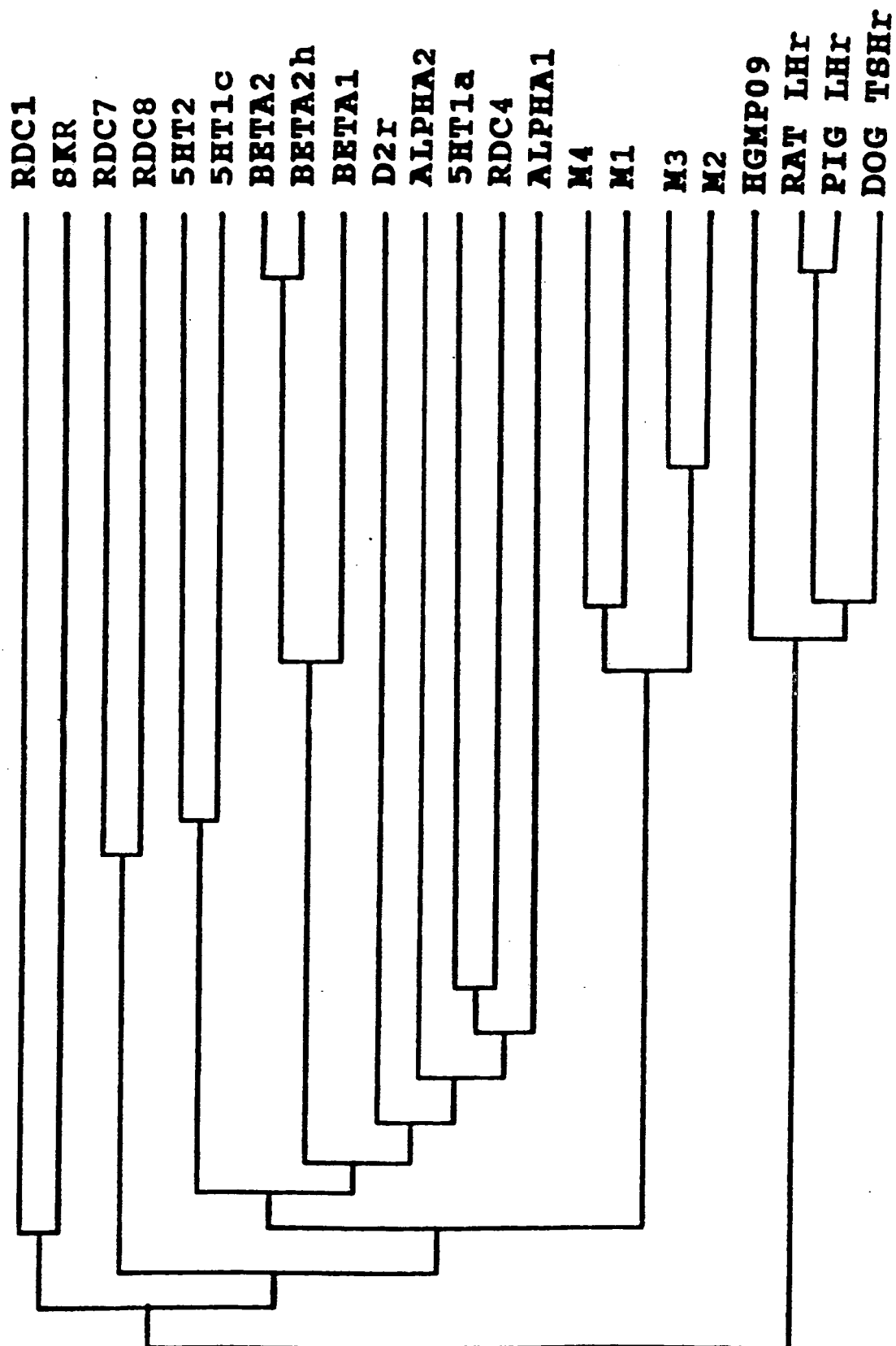


FIGURE 3a



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FIGURE 3b

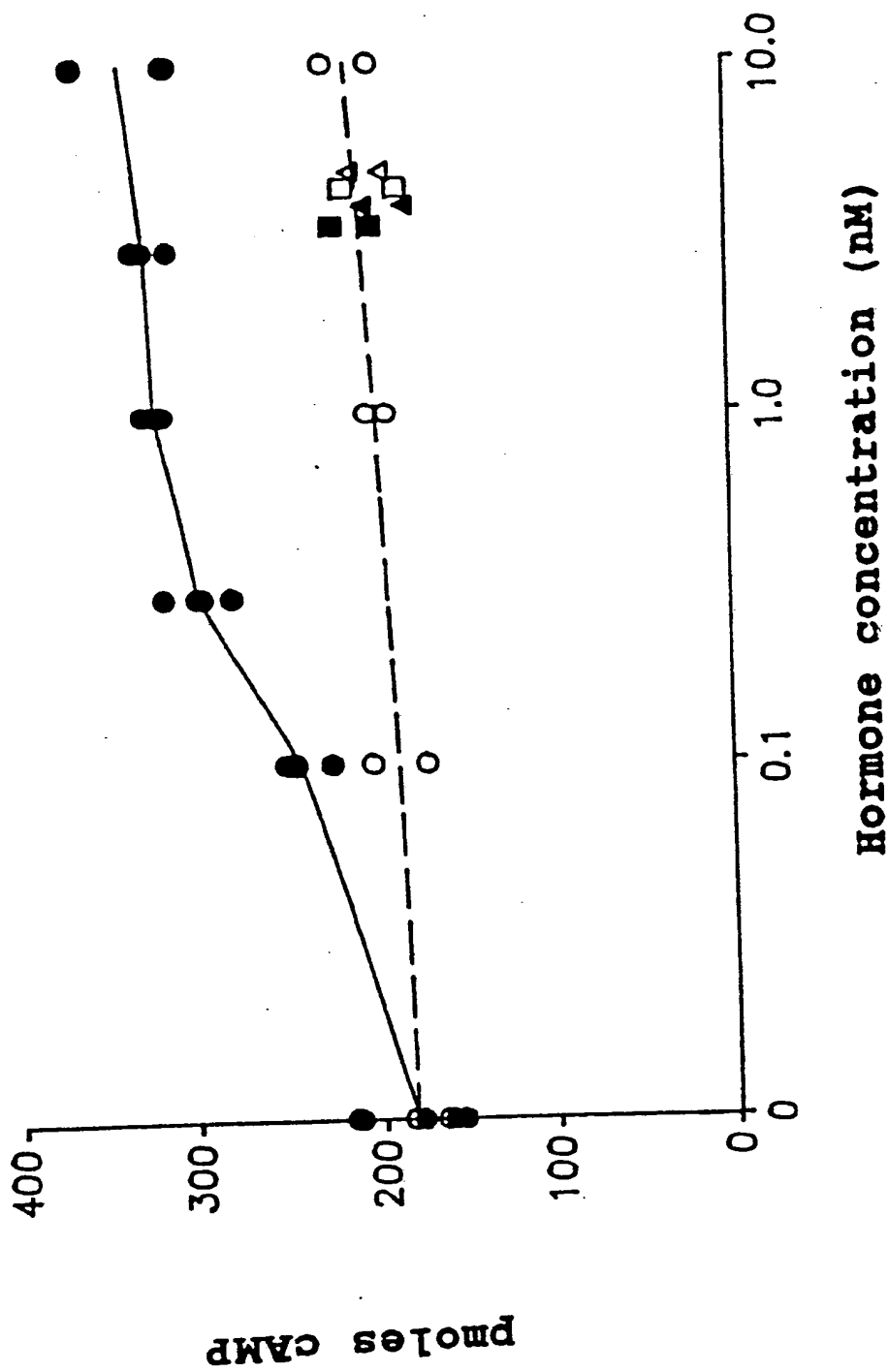


FIGURE 4

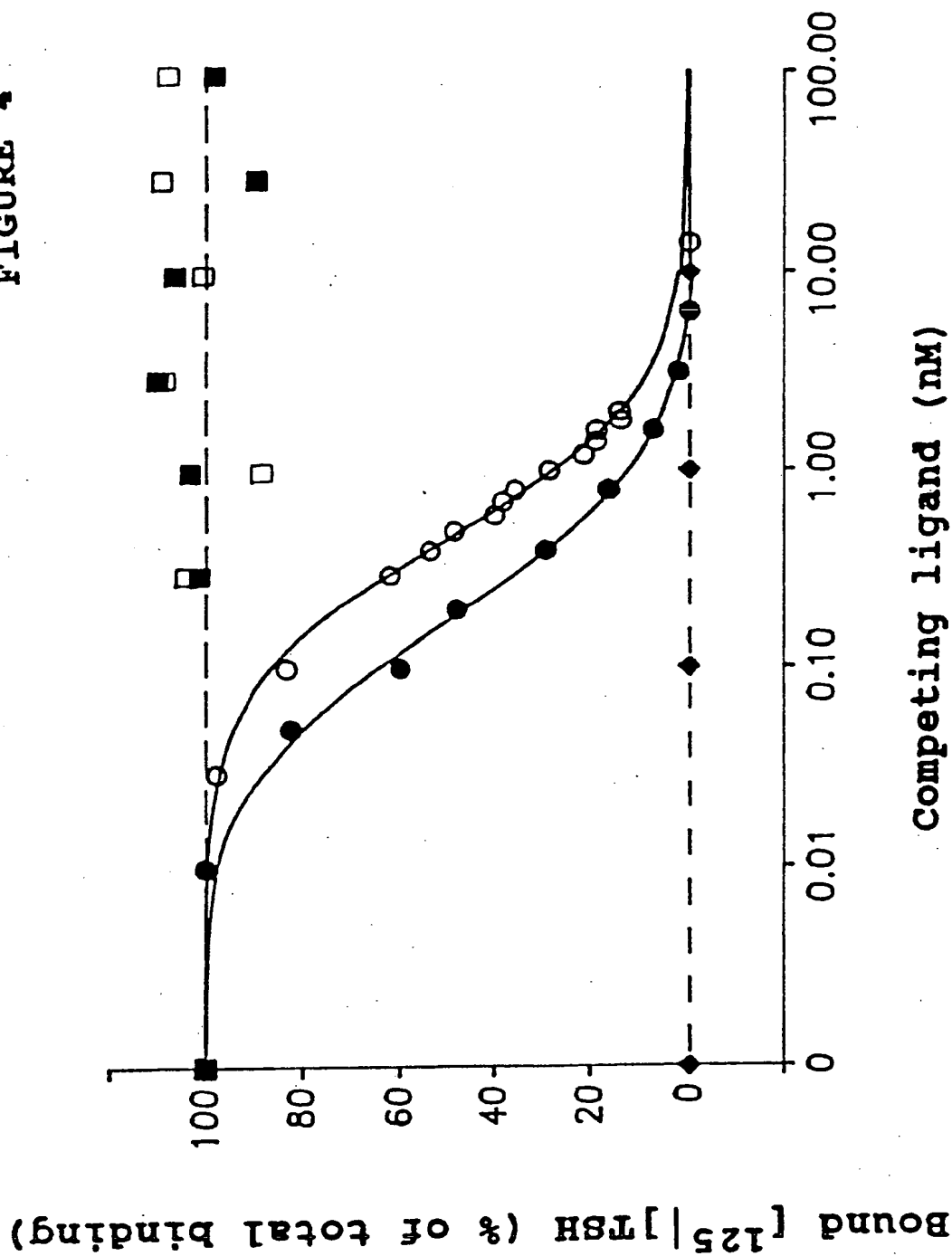


FIGURE 5

-62 CAGGCGCAGAGGGGCCCCAGACGACCGTGGAGGATGAAGAAATAGCCTTGGGACCCCTTGGAAA

1 ATGAGGGCGCGCGCCCTGCTGCACCTGGCGCTGCTTCGGCCCTGCCAGGAGCCTGGGGGGAAGGGGTGCTTCTCCCGCCCTGTGAG

91 TGCCACCAGGAGGATGACTTCAGAGTCACCTGCAAGGATATCCACCGCATCCACCCCTACCCAGCCAGCAGACTCTGAAGTTTATA

181 GAGACTCAGCTGAANAACCATTCGCCAGTCGTGCATTTTCAAAATCIGCCCAATATTTCCAGGATCTACTTGTCAATAGATGCAACTCTGCAG

271 CGGCTGGAAATCACATTCTTACAAATTAAGTAAATGACATACATAGAGATTCGGAATACCCAGAAGCTTAACATCCATAGACCCCTGAC

361 GCCCTAAAAGAGCTCCCACTCCCTGAAGTTCCCTGGCATTTTCAACACATGGACTTGGAGTATTCCTTGATGTGACCCAAAGTTTATTCCTACT

451 GATGTATCTTTATACTTGAATACAGACAACCTTACATGGCTTCCATCCCTGCCAATGCTTCCAGGGCTGTGCAATGAACCCCTG

541 ACACTGAAACTATACAACAATGGCTTTACTTCAATCCCAAGGACATGCTTCAATGGGACAAAACCTGGATGCTGTTTACCCTGAACAAGAAAT

631 AAATACCTGTCAGCTATCGACAAGATGCATTTGGAGGAGTGTACAGTGGACCAACCTTGGTGGATGCTCTTACACCAGTGTACTGCCC

721 CTGCCATCCAAAGGCCCTGGAGCATCTAAGGAGCTGATAGCAAGAAACACTTGGACTCTAAGAAACTCCCACCTTCCCTTGAGTTTCCCTT

811 CACCTTACACGGGCTGACCTTCTTATCCAAGCCACATGCTGTGCTTTAAGAAATCAGAAGAAAATCAGAGGAAATCCTTGAGTCCCTTAATG

901 TGTAAATGAAGCAGTATTCGGAGCCTGCGCCAGAGAAAATCTGTGAATACCTTGAATGGCCCTTTGACCAGGAAATATGAAGAGTATCTG

991 GGTGACAGCCATGCTGGGTACAAGGACACATCTCAGTCCAGGATACCGATAGCAATCTCATTTATATGCTTCTTCGAAGAACAAGAA

1081 GATGAGATCCTCGGTTTTGGCAGGAGCTTAAAACCCACAGGAAGACCCCTCCAGGCCCTTGATAGCCATTATGACTACACTGTGTGT

1171 GGTGGCAATGAAGACATGGTGTACTCTAAGTCAGATGAGTTCACCCCTGTGAAGACATAATGGGCTACAAGTTCTTGAGGATTGTG

1261 GTGTGGTTTGTAGTCTGCTGGCTCTCCTGGGCAATGCTTTGTCTGTATGCTCTCTTACCAGTCACATAAAATGACTGTCCACCGC

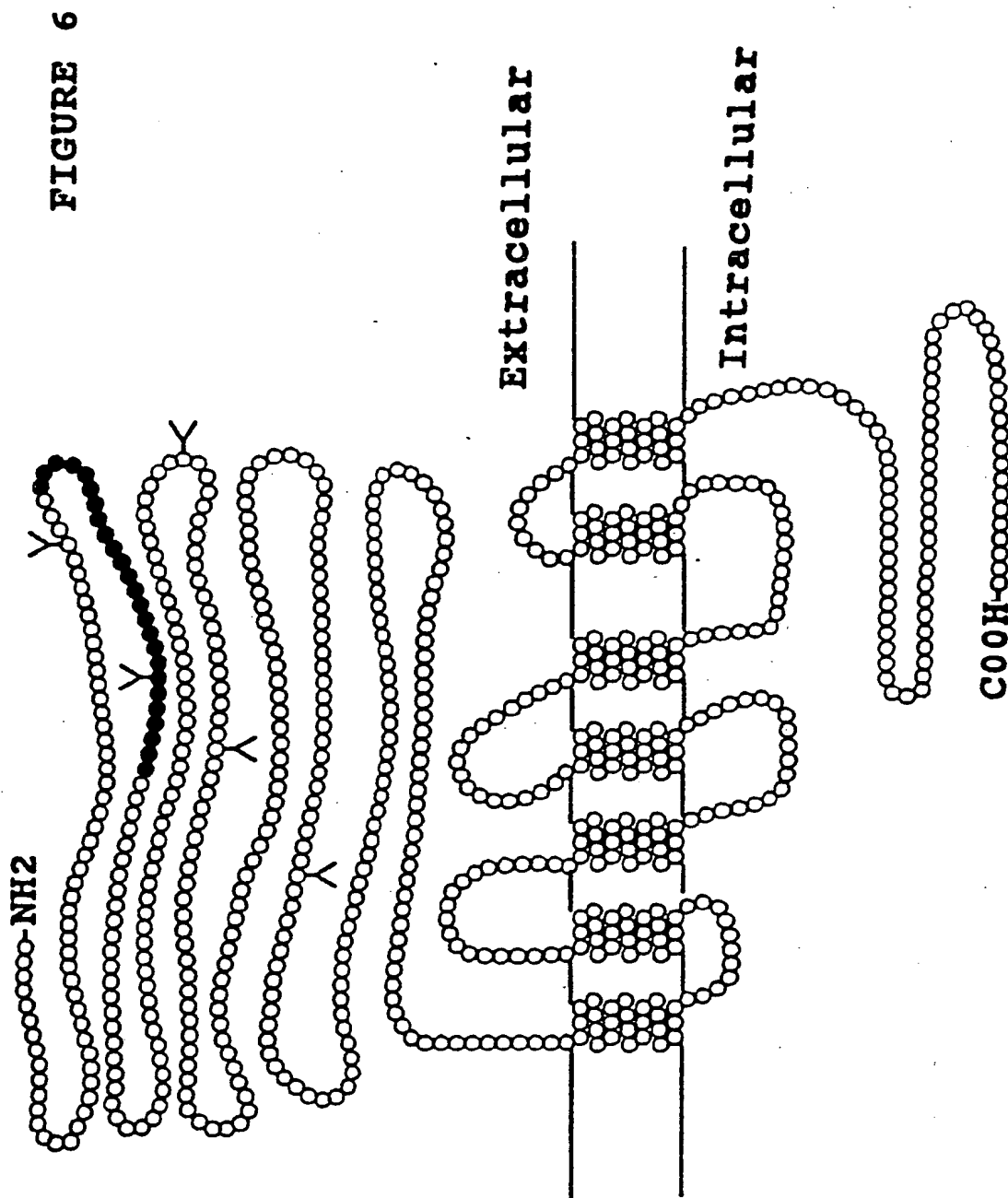
1351 TTCTCATGTGCAACTTGGCCTTTTGCAGATTTCTGCATGGGATGTATCTGCTCTCTCATCGCCTCCGTAGACCTCTACACTCATCTGAG

FIGURE 5 - SUITE 1

1441 TACTACAACCATGCCATCGACTGGCAGACAGGCCCTGGGTGTAACACAGCTGGTTTCTTCACTGTCTTTGCCAGIGAATTATCAGTGTAT
1531 AACTGACAGTCAACCCCTGGAGCGCTGGTATGCCATTACCTTCGCCATGGCTGGACAGGAAGATCGGCTCAGGCGATGCATATGCC
1621 ATCATGGT TGGGGCTGGGTTTGCTGCTTCCCTGCTGCGCCCTGCTCCCTCIGGIGGGAATAAGCAGCTATGCCAAGGTCAGCATCTGCCCTG
1711 CCCATGGACACTGAGACACCTCTTGCCCTGGCATAATATATCCTTGTTCTGTTGCTCAACATAGTTGCCCTTTATCATTTGCTGCTCCTGT
1801 TATGTGAAGATCTACATCAGTCCGAAATCCCCAGTACAAACCGGGGGACAAAGACACCAAAATGGCCAAAAGGATGGCTGTATTGATC
1891 TTCACCTGACTTCAIGTCATGGCCCCCAATCTCATCTACGCTCTGTCAGCACCTTATGAACAAGCCCTCICATCACIGTTACCAACATCCAAA
1981 ATCTTGTGTTCTCTTATCCACTTAACCTCTGTCCTCAATCCATTTCTCTATGCTATTTTCAGGAAAGCCCTCCAGAGGGAATGTTT
2071 ATCTGCTCAGCAAGTTTGGCATCTGTAAACGCCAGGCTCAGGCATACCGGGGCCAGAGGGTTTCTCCAAAGAATAGTGTGGTATTCAG
2161 ATCCAAAGGTTACCGGGACATGAGGCAAGTCTCCCCAACATGCAGGATGAGTATGAACCTGTGAAAACTCCCATCTAACCCCAAAT
2251 AAGCAGGGCCAAATCTCAAAGAGTATAACCAACAGTTCTGTGAAGCAGACCCCTATACTACGCGAGTGGCAGGTGGACTTCTAAAAATC
2341 TAGTTTCTTGAACACGTATTCCAAATTCATTATATACACAAGACAGCTGACCTAACCCCTTTCAGGIGATGTTTCATGGGGCAAAATTTCA
2431 TCTCCAAAAGGGGTAGCTCTACCACCTAATCATTACCTCCAGAGGAAGAGAGGCTACCAGCACCTTCTGAACCCCTGGTGTATATCAAG
2521 ATAACCTGACACTTCTAGAAAACCTGTTTIGATGCTAACCTGTTTAAACAACATTTGTATAAGATGTCCAACAGATATTAACTGAACCCAGGTC
2611 AACATTGAGCTTCTCACTTTCAAATAGCATTTATAGTAAAGATTCGCAATGGCAAAATGCTATTAACCTGAGTTGGTGACCACAAGATA
2701 GAATTAGCCCCATGTTGGCTTGGTCCACCTTTCATGTTCTTGGATACAAACCAAGAGAAATGGAATTCCTCGAAACTGAAAAGTCCAGCAG
2791 GATACATGCATGAAGCAGCTATTATGAGGTGAAGGGGAAAGGCTTAGCTTAGTTGTTATTTTCAGCCTCTGAAACTATATCATCTCT
2881 TCACAAGGACCTACCTGATGTGACCCCACTGTTAGGTGTTGCCAGGGGGGAAAAAACTGGCAAGATTTACGCTTATGTGGCTGAGCAA

FIGURE 5 - SUITE 2

2971 AGTAAGAATTGTTCTTGGCTAGTCTTATAGCATAAATACGTGAACCCCTAGAAATATTTCTAAGTAGCAGCAAGTGGGAATTATGAG
3061 CAGGGCACACTAAATCACACACTGATTAAATAAGCAGGGCCACAAAGGTAACTGTTGGAGCTTGGGCAAAATCACTGGGCCACTTCTAAGTC
3151 TAGAAATGAGAGAGCCTGATTGCTTCTTCAGTTTCAAAACICTAIGTATACCCCTTCCCTTAAATAATGTTCCATGACAAAAAGAAA
3241 AGCACTAAAAAGAAAAGAAAAGCACTAAGAAAGAAAAATTATTTTCCATCTTGTAGTGCAGCCACCTCTTTCCTCTTTGGAG
3331 GCTGGATAATGACCCAGGACATTCTCTTTTTTATTTTTTTCATTTTGTATTAAIGTCTGATCCATGTTGGGCTGGATCT
3421 AAATCACICTAACTAATTACTAGATCTCTACAGCTACAATTATCAGGCCAAAACAGACTCATATCACATAACAGAAATAAAAGGTGGTTT
3511 TGCAAATTTTGGTTATTCAGAGTTACTACTCTCAGTATAGATTAACTTGAAAACATTTAACTTGTCAGGGATTGGAAGCTATCAAACA
3601 CTCAGGCAAGCAACACTAAAGCTATCAAGAGAAGTTTCTTCTCTCCAAACTGCTAGCCTTTTCCAACTGTGTGATCATTTGGACATAAT
3691 CTCTATTGCCCCAATAGTGTCTCTTACTTAAATGGTTAGGATCAATCTTTAATATAGACGTACTCTTCAGATTACCTGTCAAAACAGT
3781 CCCTTAATTTCTCCCAAGCAGAGATGGCATTGTGCTTCTCAATGTTCAAGCACACCAAGGAATTAGAAGCATTTGTTGTTCAGTC
3871 TGTGGAGTAGGGTTACIGGGCCCAATGCCCCCCCCCAGAGATGGTCCCCCAACCCACCTAGGATATCCCAATAGCAATACCCCATTT
3961 CTGATTATCATTGAGATTGGACATCTTAGTAGAAATATTATACACACTCGAAATCAIGACTTATCCACCAGTTCACCTGTAACATAAC
4051 TAAACAGTTGTGTATCGTTTGGCATGTGTTTCTCACCTGTGACATTTTGAAATAGTACATCTGATAATGTAATTTATCTTAAGTAGTT
4141 GAAATAACACTTTGGAAACCGTCTAGAAAAGTAACCTCAACACAATTTGTTACTAAAAATTGCAATTCACAACAIGAAAATAAATTTCTTC
4231 CTATGAATGATTGTGCTGAGTCTACAGTATGGCAATTTTGTAATTTGTGAGCTTCTTTTAAATGTTACCGTTAATGTTACCAACIGAA
4321 GACAGGGAAAAAACAACATGGCAAAATTTGCTAA



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FIGURE 7

-20 MRPPPLLHLALLALPRSLG
1 GKGCPSPPCECHQEDDFRVCKDIHRIPTLPPSTQT
37 LKF-IETQLKTIPSRFSLNLPNISR
61 IYLSIDATLQRLSHSFYNLSKMTH <
86 IEIRNTRSLTSIDPDALKELPLKF
111 LGIFNTGLGVFPDVTKVYSTDVFFI
136 LEITDNPYMASIPANAFQGLCNETL
161 TLKLYNNGFTSIQGHAFNGTKLDAV
186 YLN-KNKYLSAIDKDAFGGVYSGPT
210 LLDVSYTSVTALPSKGLEHLKELIA
235 RNTWTLKKLP-L-SLSFLHLTRADL...
Cons L^NLIXX^NXXIX^NSIP^ASX^AFXGLXXXXX
I^TT^TALD^SS

FIGURE 8

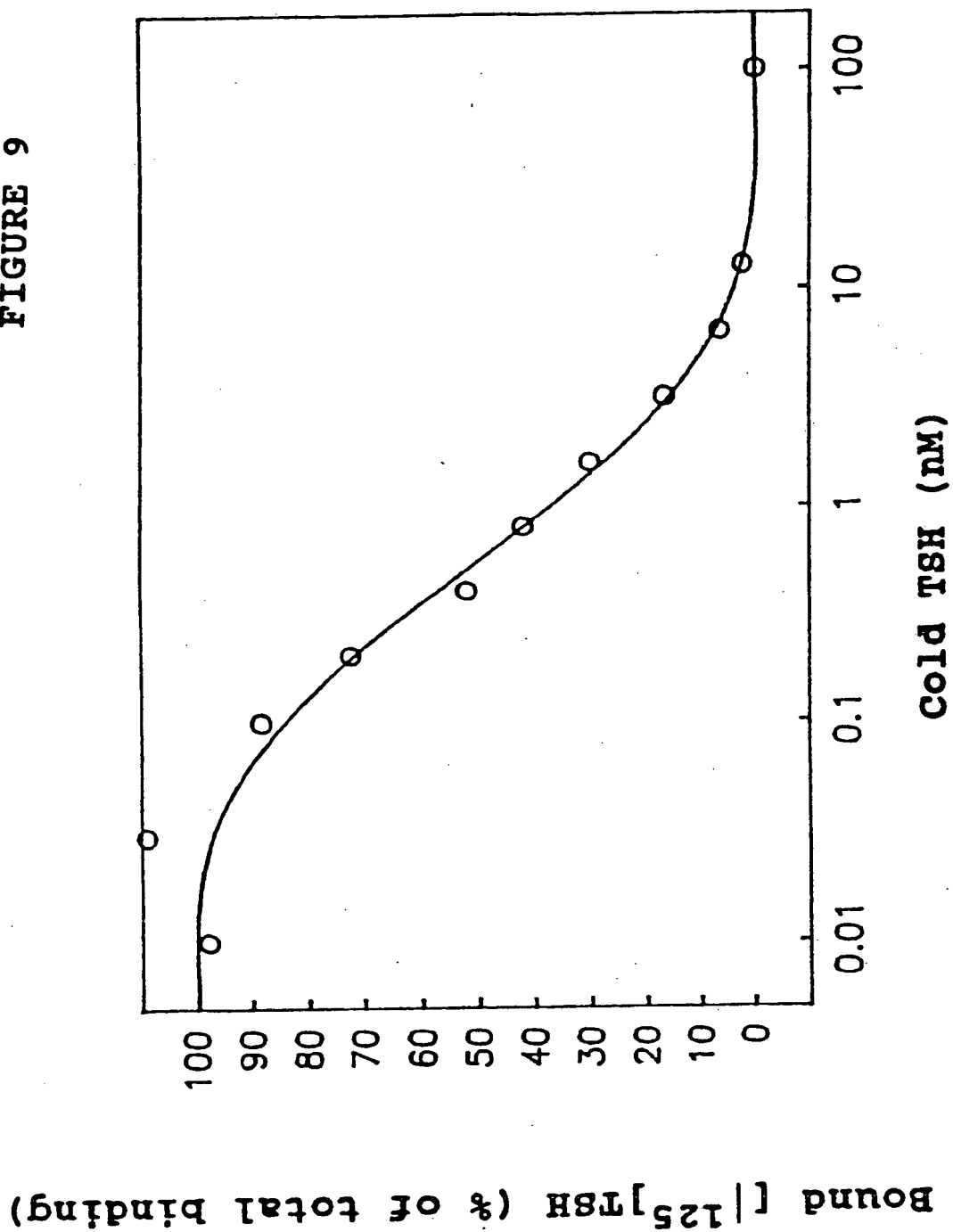
Comparison of human and dog TSH receptor sequences

	-20	1	20	40
Human TSHR	MRPADLLQLVLLDLPRDLGGMGCSPPCECHQEEDFRVTCKDIQRIPSLPPSTQTLKLI			
Dog TSHR	PP H A A S K P D H T F			
	60	80	100	
Human TSHR	ETHLRTPSHAFSNLPNLSRIYVSIDLTLQQLSHSFYNLSKVTHIEIRNTRNLTYIDPD			
Dog TSHR	Q K R L A R M S S			
	120	140	160	
Human TSHR	ALKELPLLKFLGIFNTGLKMFDPDLTKVYSTDIFILEITDNPYMTSIPVNAFQGLCNETL			
Dog TSHR	GV V V A A			
	180	200	220	
Human TSHR	TLKLYNNGFTSVQGYAFNGTKLDVAVLNKNKYLTVIDKDAFGGVYSGPSLLDVTSQTSVTA			
Dog TSHR	I H SA T Y			
	240	260	280	
Human TSHR	LPSKGLEHLKELIARNTWTLKKLPLSLSLFLHLTRADLSYP SHCCAFKNQKIRGILES LM			
Dog TSHR				
	300	320	340	
Human TSHR	CNESSMQSLRQKSVNALNSPLHQEYEENLGDSIVGYKEKSKFQDTHNNAHYVVFEEQE			
Dog TSHR	IR T G FD Y HA DN Q DS S			

FIGURE 8 - SUITE 1

Human TSHR	360	380	400
Dog TSHR	DEIIGFGQELKNPQEETLQAFD	SHYDYTICGDS	EDMVCTPKSDEFN
	L	V	GN
Human TSHR	420	440	460
Dog TSHR	VWFVSLALLGNVFEVLLILLTSHYKLNVPFLMCNLAFAFCMGMYLLLIASVDLYTHSE	II	
	IV	T	
Human TSHR	480	500	520
Dog TSHR	YYNHAIDWQTGPGCNTAGFFTVFASELSVYTLTVITLERWYAITFAMRLDRKIRLRHACA	III	Y
Human TSHR	540	560	580
Dog TSHR	IMVGWVCCFLALLPLVGISSYAKVSI	CLPMDTETPLALAYIVFVLT	LNIVAFVIVCCC
		IL	L I S
Human TSHR	600	620	640
Dog TSHR	YVKIYITVRNPQYNPGDKDTKIAKRM	AVLI	FTDFICMAPI
		M	SFYALSAILNKPLITVSNSK
Human TSHR	660	680	700
Dog TSHR	ILLVLFYPLNSCANPFLYAI	FTKAFQRDV	FILLSKFGICKRQAQAYRGQ
	VII		VPKNS
Human TSHR	720	740	
Dog TSHR	VQKVTHDMRQGLHNMEDVYELIENSHLTPKKQ	QI	SEYMQTVL
	I	R S P Q E L	N K N

FIGURE 9



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FIGURE 10

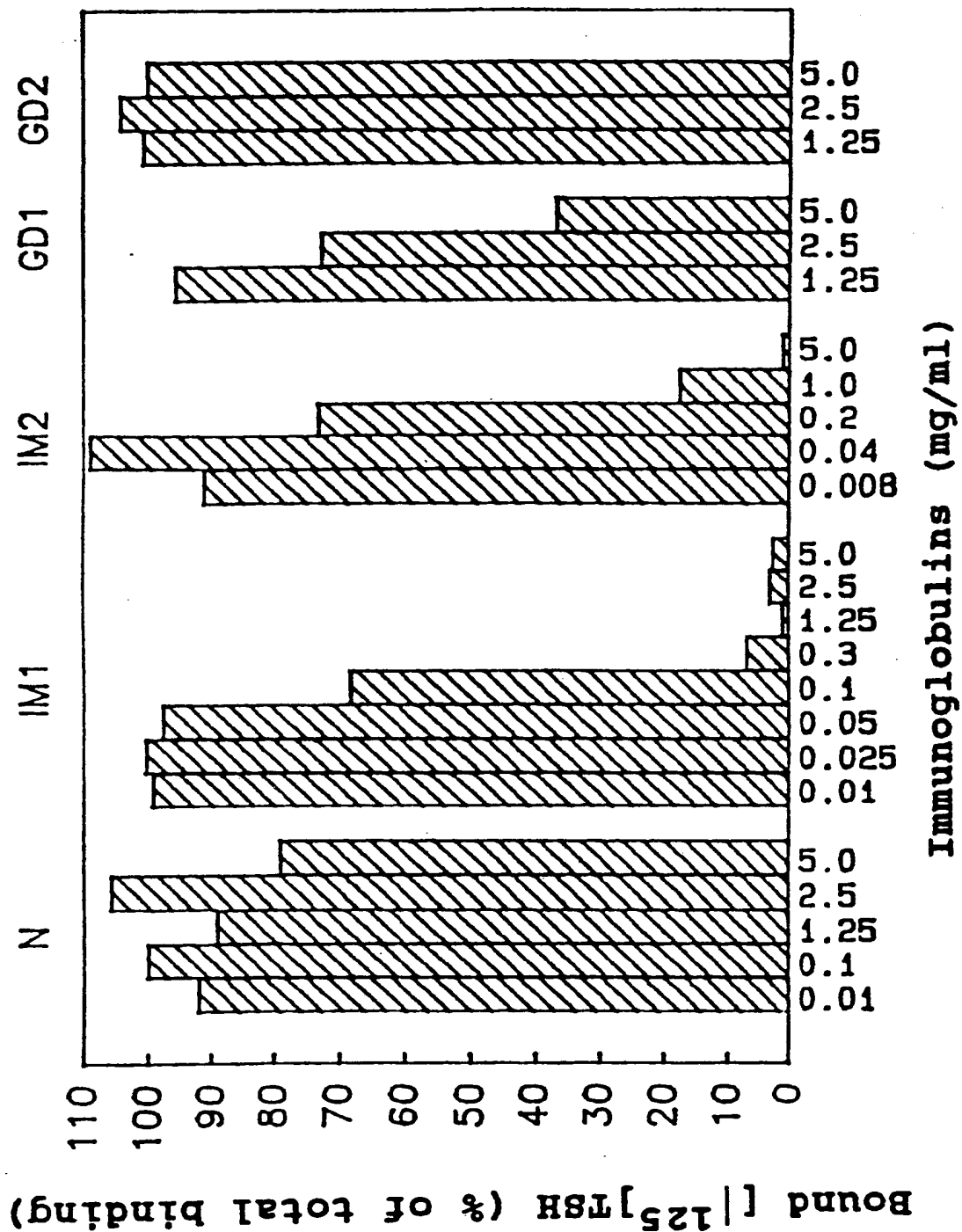


FIGURE 11

-20 1 20 40
 MRPADLLQLVLLDLPRDLGGMGSSPPCECHQEEDFRVTCCKDIQRIPSLPPSTQTLKLI
 PP H A A S K P D H T F
 60 80 100
 ETHLR TIPSHAFSNLPNISRIYVSIDLTLQQLESHSHFYNSKVT HIEIRNTRNLTYIDPD
 Q K R L A R M S S
 120 140 160
 ALKELPLLKFLGIFNTGLKMFDPDLTKVYSTDIFFILEITDNPYMTSIPVNAFQGLCNETL
 GV V V A A
 180 200 220
 TLKLYNNGFTSVQGYAFNGTKLDVYLNKNKYLTVIDKDAFGGVYSGPSLLDVSQTSVTA
 I H SA T Y
 240 260 280
 LPSKGLEHLKELIARNTWTLLKPLSLFLHLTRADLSYPSHCCAFKNQKIRGILESLM
 300 320 340
 CNESSMQSLRQKSVNALNSPLHQEYEENLGDSIVGYKEKSKFQDTHNNAHYVFFEEQE
 IR T G FD Y HA DN Q DS S
 360 380 400
 DEIIGCGQELKNPQEETLQAFDSDHYDTICGDSMDVCTPKSDEFNCPCEDIMGYKFLRIV
 L V GN

FIGURE 11 - SUITE 1

I 420 440 460
 VWFVSLALLGNVFLILLTSHYKLNVPRTFLMCNLAFAFCMGMYLLLIASVDLYTHSE
 IV T I I IH K Q
 YYNHAIDWQTGPGCNTAGFFTFVFASELSVYTLTVITLERWYAITFAMRLDRKIRLRHACA
 H Y A DA HT H Q C VO YS A
 480 500 520
 IV 540 560 580
 IMVGGWCCFLALLPLVGISSYAKVSI CLPMDTETPLALAYIVFVLTNIVAFVIVCCC
 V M IFA AA F IF M IDS SLQ VILL L VL I S
 YVKIYITVRNPQYNPGDKDTKIAKRMAVLFTDFICMAPISFYALSAILNKPLITVSNSK
 600 620 640
 VI LM T
 VII 660 680 700
 ILLVLFYPLNSCANPFYAIFTKAFQRDVFILLSKFGICKRQAQAYRGQRVPPKNSTD IQ
 S AG
 720 740
 VQKVTHDMRQGLHNMEDVYELIENSHLTPKKQGI SEEYMQTVL
 I R S P Q E L K N

FIGURE 12

5' AGGCAGCAGTTTCCTCCTGGGACCTGATGGCTCCAGATCACTATCTTGGGCCCAGACTTTCTGGAGCTG 70
80 90 100 110 120 130 140
AATCTCCAGTTGCCTCGGAGCCTCCTCAGACTCAGTGTGGCCAGAAATGGTGCTTCCCTCCCTCGGG
150 160 170 180 190 200 210
CCTGCCCTTCTGCCTCCTTCTGCACCCCTGAGATGGTCATCAGCTTTTCTCCCACTGCTGCCCTGTATGCA
220 230 240 250 260 270 280
GGGAAGCCCTGCCCTGTGCTGTATCTGTAGTACTTCTTGAATGTGTTTCTTCTCCCAAGCCAGAGCT
290 300 310 320 330 340 350
GAGAAATGAGGCGATTTCGGAGGATGGAGAAATAGCCCCGAGTCCCGTGGAATAAGGCCCAGGACTTG
360 370 380 390 400 410 420
CTGCAGCTGGTGTGCTGCACCTGCCCAAGGACCTGGGCGGAATGGGGTGTTCGTCTCCACCCCTGCG
430 440 450 460 470 480 490
AGTGCCATCAGGAGGAGGACTTCAGAGTCACTGCAAGGATATTCAACGCAATCCCAAGCTTACCGCCAG
500 510 520 530 540 550 560
TACGCAGACTCTGAAGCTTATTGAGACTCACCTGAGAACTATTCCAAGTCATGCATTTTCTAATCTGCCC
570 580 590 600 610 620 630
AATATTTCCAGAACTACGTATCTATAGATCTGACTCTGCAGCAGCTGGAATCACACTCCTTCTACAATT

22/26

FIGURE 12 - SUITE 1

640 650 660 670 680 690 700
TGAGTAAAGTEACTCACATAGAAATTTCGGAATACCGAACTTAACCTTACATAGACCCCTGATGCCCTCAA
710 720 730 740 750 760 770
AGAGCTCCCCCTCCTAAAGTTCCTTGGCATTTCACACACTGGACTTAAATGTTCCCTGACCTGACCAA
780 790 800 810 820 830 840
GTTTATTCCACTGATATATTCTTTATACCTTGAAATTACAGACACCCCTTACATGACGTCAATCCCTGTGA
850 860 870 880 890 900 910
ATGCTTTTCAGGGACTATGCAATGAAACCTTGACACTGAAGCTGTACAACAATGGCTTTACTTCAGTCCA
920 930 940 950 960 970 980
AGGATATGCTTTCAATGGGACAAAGCTGGATGCTGTTTACCTAAACAAGAATAAATACCTGACAGTTATT
990 1000 1010 1020 1030 1040 1050
GACAAAGATGCATTTGGAGGAGTATACAGTGGACCAAGCTTGCTGGACGTGTCTCAAACCAAGTGTCACTG
1060 1070 1080 1090 1100 1110 1120
CCCTTCCATCCAAAGGCCCTGGAGCACCTGAAAGAACTGATAGCAAGAAACACCTGGACTCTTAAGAAACT
1130 1140 1150 1160 1170 1180 1190
TCCACTTTCCCTTGAGTTTCCCTTACCTCACACGGGCTGACCTTTCTTACCCAAGCCACTGCTGTGCTTTT

23/26

FIGURE 12 - SUITE 2

1200 1210 1220 1230 1240 1250 1260
AAGAATCAGAGAAATCAGAGGAATCCTTGAGTCCTTGATGTGTAATGAGAGCAGTATGCAGAGCTTGC
1270 1280 1290 1300 1310 1320 1330
GCCAGAGAAATCTGTGAATGCCCTTGAATAGCCCCCTCCACCAGGAATATGAAGAGAACTCTGGGTGACAG
1340 1350 1360 1370 1380 1390 1400
CATTGTTGGGTACAAGGAAAGTCCCAAGTTCCAGGATACCTCATACAACGCTCATTATTACGTCTTCTTT
1410 1420 1430 1440 1450 1460 1470
GAAGAACAGAGGATGAGATCATTGGTTTTTGCCAGGAGCTCAAAAACCCAGGAGAGAGACTCTACAAG
1480 1490 1500 1510 1520 1530 1540
CTTTTGACAGCCATTATGACTACACCATATGTGGGGACAGTGAAGACATGGTGTGTACCCCAAGTCCGA
1550 1560 1570 1580 1590 1600 1610
TGAGTTCAACCCGTGTGAAGACATAATGGGCTACAAGTTCCTGAGAAATTGTGGTGTGGTTCTGTTAGTCTG
1620 1630 1640 1650 1660 1670 1680
CTGGCTCTCCTGGGCAATGTCTTTGTCTCTGCTTATTCTCTCACCAGCCACTACAACTGAACGTCCCCC
1690 1700 1710 1720 1730 1740 1750
GCTTTCTCATGTGCAACCTGGCCCTTTGCGGATTTCTGCATGGGGATGTACCTGCTCCTCATCGCCTCTGT

FIGURE 12 - SUITE 3

1760 1770 1780 1790 1800 1810 1820
AGACCTCTACACTCACTCTGAGTACTACAACCATGCCATCGACTGGCAGACAGGCCCTGGGTGCAACACG
1830 1840 1850 1860 1870 1880 1890
GCTGGTTTCTTCACTGTCTTTGCAAGCGAGTTATCGGTGTATACGCTGACGGTCAACCCCTGGAGCGCT
1900 1910 1920 1930 1940 1950 1960
GGTATGCCATCACCTTCGCCATGCGCCTGGACCGGAAGATCCGCCTCAGGACGCGCATGTGCCATCATGGT
1970 1980 1990 2000 2010 2020 2030
TGGGGGCTGGGTTTGCTGCTTCTTCCTTCGCCCCCTGCTTCCTTTGGTGGGAATAAGTAGCTATGCCAAAGTC
2040 2050 2060 2070 2080 2090 2100
AGTATCTGCCCTGCCCATGGACACCGAGACCCCTCTTGTCTGCGCATATATTGTTTTTGTCTGACGCTCA
2110 2120 2130 2140 2150 2160 2170
ACATAGTTGCCCTTCGTCTCGTCTGCTGTTATGTGAAGATCTACATCACAGTCCGAAATCCGCAGTA
2180 2190 2200 2210 2220 2230 2240
CAACCCAGGGGACAAAGATACCAAAATTGCCAAGAGGATGGCTGTGTTGATCTTCACCGACTTCATATGC
2250 2260 2270 2280 2290 2300 2310
ATGGCCCCAATCTCATTTCTATGCTCTGTGCGCAATTCTGAACAAGCCCTCTCATCACTGTTAGCAACTCCA
2320 2330 2340 2350 2360 2370 2380
AAATCTTGCTGGTACTCTTCTATCCACTTAACCTCCTGTGCGCAATCCATTCTCTATGCTATTTTCACCAA

FIGURE 12 - SUITE 4

2390 2400 2410 2420 2430 2440 2450
GGCCTTCCAGAGGATGTGTTTCATCCTACTCAGCAAGTTTGGCATCTGTAAACGCCAGGCTCAGGCATAC
2460 2470 2480 2490 2500 2510 2520
CGGGGCGAGAGGGTTCCTCCAAGACAGCAGCTGATATTCAGGTTCAAAGGTTACCCACGACATGAGGC
2530 2540 2550 2560 2570 2580 2590
AGGGTCTCCACAACATGGAAGATGTCTATGAAGTGAAGTCCCATCTAACCCCAAAGAGCAAGG
2600 2610 2620 2630 2640 2650 2660
CCAAATCTCAGAGAGTATATGCAACGGTTTTGTAGTTAACACTACACTACACAATGCTAGGGGAA
2670 2680 2690 2700 2710 2720 2730
CTTACAAAATAAGTTTCTTGAATATGCATTCCCAATCCCATGACACCCCAACACATAGCTGCCCTCAC
2740 2750 2760 2770 2780 2790 2800
TCTTGTGCGCGATGTTTCAATGTTTCATGGGCAAGAGTTTATCTCTGGAGAGTGATTAGTATTAAACC
2810 2820 2830 2840 2850 2860 2870
TAATCATTGCCCCCAAGAGGAGTTAGGCTACCCAGCATATTTGAATGCCAGGTTGAAATCAAAATAATCT
2880 2890 2900 2910 2920 2930 2940
ACACTATCTAGAGACTTTTCTTGATGCCAAGTCCAGAGATGTCATTGTGTAGGATGTTTCAGTAAATATTA
2950 2960 2970 2980 2990 3000 3010
ACTGAGCTATGTCAATATAGAGCTTCTCAGTTTTGTATACATTTTCATATAAGATTTCAGCAAAATGGAA
3020 3030 3040 3050 3060 3070 3080
AATGCTATTAAATTTGGTTGGTGACCAAGATAAATCAGTCCACGTTGGCTCAGTTCAACTAGATGTT

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26/26

FIGURE 12 - SUITE 5

3090 3100 3110 3120 3130 3140 3150
CCCTGATACAAAGAGAACTTGATTTCCTTAAACTGAAAAGCCAAACACAGCTAGCTGTGCATACAGAAA
3160 3170 3180 3190 3200 3210 3220
CAGCTATTATGAGACATGAAGGAGGGTAAGAATTAGCTTTAAGTTTGTGCTTTGTTGTTTTTTA
3230 3240 3250 3260 3270 3280 3290
ACTCAACCTATTATCATCTCTTCACAAGAATCCACCTGATGTGACCAAGCTATTATGTTGCCTGGAA
3300 3310 3320 3330 3340 3350 3360
AACTGGCAAGATTTGAGCTTATGTGGCCTAGCAAACTAAGAAATTGCTCTTCTTGGCCAGCCTCATAGCA
3370 3380 3390 3400 3410 3420 3430
TAAAGATGTGAACCTAGGAAGTCTTTCTGAGTAGCAATAAGTGGAATTATGGGCAGAGCACACTCAA
3440 3450 3460 3470 3480 3490 3500
TCCCCTGTTGATTAAATAAACAGGCTGGACACTAATTAACCTATGGGACTTAATCTGTAGAAATGAAGGA
3510 3520 3530 3540 3550 3560 3570
GTCCAATAGCTTCTTCCAAATTTTAAACTCTAGTACATCCCCTTCCCTCAAATATATTTCTAAGATAA
3580 3590 3600 3610 3620 3630 3640
AGAGAAAGAGAGCACTAAGTAGAATCTGTTTTTCCCTATTTTGTAGGGCTGCTGACTCCTAGTCCT
3650 3660 3670 3680 3690 3700 3710
TGAAGCCTAGACACATGACCCAGGAAATTTTCCCTTTGTTTCACTTTTGATTATGATGCTGAGGCCAAAAA 3'



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(71) Applicant (for all designated States except US): HENNING
BERLIN GMBH CHEMIE- UND PHARMAWERK
[DE/DE]; Komturstr. 58-62, D-1000 Berlin 42 (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): PARMENTIER, Marc
[BE/BE]; Avenue Minerve 21 Bte 81, B-1190 Brussels
(BE). LIBERT, Frederic [BE/BE]; Brusselbaan 115, B-
1600 St-Pieters-Leeuw (BE). DUMONT, Jacques [BE/
BE]; Chemin du Chêne-au-Rénard 32, B-1328 Ohain
(BE). VASSART, Gilbert [BE/BE]; Avenue Lambeau
113, B-1200 Brussels (BE).

(74) Agent: ANDRAE, Steffen; Patentanwälte Andrae, Flach,
Haug, Kneissl, Balanstr. 55, D-8000 München 90 (DE).

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(57) Abstract

The invention concerns a polypeptide possessing
thyrotropin receptor activity, characterised in that it com-
prises the amino acid sequence (shown in Fig. 11), or a
fragment thereof, or an amino acid sequence derived from
this sequence by substitution or deletion of any of the am-
ino acid residues (indicated in Fig. 11), or by insertion of
additional amino acid residues.

Comparison of human and dog TSH receptor sequences

	-20	1	20	40
Human TSHR	MRPADLLQLVLLLDLPRLDGGHGCCSPPCCHQZEDFRVTCQDIQRIPLPSPSTOTLRLI			
Dog TSHR	PP H A A S K P D H T F			
	60	80	100	
Human TSHR	ETHLRTIPSHAFSNLPHISRIYVSIDLTLQQLSHSFYHLSKVTHIEIRNTANLTYIDPD			
Dog TSHR	Q K R L A R M S S			
	120	140	160	
Human TSHR	ALKELPLKFLGIFNTGLKMFPLTKVYSTDIFFILEITDNPTMTSIPVNAFOGLCHETL			
Dog TSHR	GV V V A A			
	180	200	220	
Human TSHR	TLKLYNNGFTSVQGYAFNGTKLDAVTLNKNKYLTVIDKDAFGCVYSGPSLLDVSQTSVTA			
Dog TSHR	I H SA T Y			
	240	260	280	
Human TSHR	LPSKGLEHLKELTARNTWTLKKPLSLSLHLTRADLSYPSHCCAFKNQKKIRGILESILM			
Dog TSHR				
	300	320	340	
Human TSHR	CHLSHQSLSLRQKSVNALMSPLHQEYENLGDIVGTRKSKPQDTHNNHMYVYVFEQZ			
Dog TSHR	IR T G PD Y HA DN Q DS S			


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INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 90/02154

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁵ : C 12 N 15/12, C 12 P 21/02, G 01 N 33/76, C 12 P 21/08, C 07 K 13/00, C 12 N 15/62		
II. FIELDS SEARCHED		
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Classification System	Classification Symbols	
IPC ⁵	C 12 N, C 12 P, C 07 K, G 01 N	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	Endocrinology, vol. 110, no. 4, 1982 The Endocrine Society, (Springfield, US) Y. Koizumi et al.: "Solubilization, purification, and partial characteri- zation of thyrotropin receptor from bovine and human thyroid glands", pages 1381-1391, see the whole document --	1-3,5
X	Clinical Research, vol. 29, no. 2, 1981 (New York, US) M.N. Islam et al.: "Purification of the human thyrotropin receptor", page 294A, see the whole article --	1-3,5
A	64th Meeting of the American Thyroid Association, abstract T-51, 1989, Frazier-Seabrook et al.: "Isolation of a thyroid-specific cDNA with over 80% amino acid homology with the luteinizing hormone receptor", see the whole abstract --	1-3,5-8 ./.
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
7th June 1991	16.08.91	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE		
		Mme Dagmar FRANK

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	Science, vol. 246, 22 December 1989 (Washington, US) M. Parmentier et al.: "Molecular cloning of the thyrotropin receptor", pages 1620-1622, see the whole article --	1-3,5-8
P,X	Biochemical and Biophysical Research Communications, vol. 165, no. 3, 29 December 1989, Academic Press, Inc., (Duluth, MN, US) F. Libert et al.: "Cloning, sequencing and expression of the human thyrotropin (TSH) receptor. Evidence for binding of autoantibodies", pages 1250-1255 see the whole article --	1-3,5-8
P,X	Biochemical and Biophysical Research Communications, vol. 165, no. 3, 29 December 1989, Academic Press, Inc. (Duluth, MN, US) Y. Nagayama et al.: "Molecular cloning, sequence and functional expression of the cDNA for the human thyrotropin receptor", pages 1184-1190, see the whole document --	1-3,5-8
X	Proceedings of the National Academy of Sciences, vol. 79, November 1982, Medical Sciences, (Washington, US) W.A. Valente et al.: "Monoclonal antibodies to the thyrotropin receptor: stimulating and blocking antibodies derived from the lymphocytes of patients with Graves Disease", pages 6680-6684, see the whole article --	12
X	WO, A, 87/05703 (BOEHRINGER MANNHEIM GMBH) 24 September 1987 see page 13, lines 1-16 --	1-3,5,12
X	Endocrinology, vol. 122, no. 6, 1988 The Endocrine Society (Springfield, US) B.L. Hill et al.: "Monoclonal antibodies to the thyrotropin receptor raised by an autoantiidiotypic protocol and their relationship to monoclonal auto-antibodies from Graves patients", pages 2840-2850 see the whole article --	1-3,5,12
A		15 ./. --

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	Chemical Abstracts, vol. 108, 1988 (Columbus, Ohio, US) see page 349, abstract no. 71722w, & HU, A, 41905 (G. BAKO et al.) 28 May 1987 --	1-3,5,9,13, 16
A	Clinical Chemistry, vol. 32, no. 7, 1986 (New York, US) C. Massart et al.: "Assays for thyrotropin receptor binding and thyroid-stimulating antibodies in sera from patients with Graves' disease", pages 1332- 1335, see page 1333 --	9,13
A	Radioimmunoassay and Related Procedures in Medicine 1982, Proceedings of a sym- posium Vienna 21-25 June 1982 P. Kotulla et al.: "Radioligand receptor assay (RRA) for thyroid- stimulating hormone (TSH) using triton X-100 solubilized receptor preparation" pages 297-305, see the whole document --	1-3,5,13,9, 16
A	Science, vol. 245, 4 August 1989 (Washington, US) H. Loosfelt et al.: "Cloning and sequencing of porcine LH-hCG receptor cDNA: variants lacking transmembrane domain", pages 525-527, see the whole document --	
P,X	Molecular and Cellular Endocrinology, vol. 73, no. 1, 1 October 1990, Elsevier Scientific Publishers Ireland Ltd. M. Ludgate et al.: "Use of the recom- binant human thyrotropin receptor (TSH-R) expressed in mammalian cell lines to assay TSH-R autoantibodies", pages R13-R18, see the whole article -----	9,10,11,16,17

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VL ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This international Searching Authority found multiple inventions in this international application as follows:

Please see attached sheet!

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the international Searching Authority did not invite payment of any additional fee.

Remark on Protest

☒ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210 (supplemental sheet (2))

1. Claims 5 (completely) and 1,2,3,6,7,8 (partially)
2. Claims 1,2,3,6,7-17 (partially)
3. Claims 4 (completely) and 6,8-13 (partially)
4. Claims 9,10,11,16,17 (partially)
5. Claims 12-15 (partially)
6. Claim 13 (partially)

For further information please see form PCT/ISA/206 dated 10-04-1991.

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